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the ROCHE REVIEW *of enrichment requirements*

PRODUCT	Thiamine (B_1)		Riboflavin (B_2)		Niacin		Iron	
	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.
Enriched BREAD, or other baked products	1.1	1.8	0.7	1.6	10.0	15.0	8.0	12.5
Enriched FLOUR[1]	2.0	2.5	1.2	1.5	16.0	20.0	13.0	16.5
Enriched FARINA[2]	1.66	—	1.2	—	6.0	—	6.0	—
Enriched MACARONI products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched NOODLE products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched CORN MEALS	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched CORN GRITS[4]	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched MILLED WHITE RICE[5]	2.0	—	*		16.0	—	13.0	—

ALL FIGURES REPRESENT MILLIGRAMS PER POUND

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EFFECT OF VARIETY ON THE MILLING AND BAKING QUALITY OF BREAD AND DURUM WHEAT FLOUR BLENDS[1,2]

R. H. HARRIS, L. D. SIBBITT, and G. M. SCOTT[3]

ABSTRACT

Durum wheat was more difficult to mill than bread wheat, particularly hard red spring varieties, and the flour had higher ash content. For baking, durum flour produced loaves of inferior volume, grain and texture, and crumb color. The absorption was also lower than for hard red spring wheat but better than for soft white wheat. Kubanka was best among the durums and also superior to Golden except for crumb color. In mixing properties the durum flours generally were markedly inferior to hard red spring flours; however, Kubanka was much better than the other durums. In diastatic activity they were greatly above the others.

When blended with hard red spring wheat flour by variety, durum flour tended to decrease baking quality significantly above 20%, and mixogram properties above 50%, concentration. For Golden there was generally no change in loaf volume except with Kubanka which consistently improved the loaf volume. Durum flour generally improved the mixogram pattern of Golden flour even when present in relatively minor proportions, the loaf volume with Kubanka exceeding that of the original flours. The diastatic power of the blends increased directly with durum concentration.

Harris and Sibbitt (4) have briefly reviewed the literature on the baking quality of durum wheat flour. These reports revealed that durum flour is utilized to some extent in breadmaking, particularly in Europe, although laboratory tests showed that it usually produces more yellow and smaller loaves than bread baked from bread wheat flours alone. The same characteristics are found to a lesser extent in bread made from blends of hard red spring and durum wheat flours. The ash content and diastatic activity are higher in durum wheat flour, because of the severe grinding required in milling. Durum flour was also used for making experimental biscuits and rolls, producing more yellow products than those made from *vulgare* wheat flour

[1] Manuscript received April 14, 1952.
[2] Published with the approval of the Director of the Station.

[3] Department of Cereal Technology, North Dakota Agricultural Experiment Station.

Harris and Sibbitt found that durum flour below a concentration of 20% in blends with *vulgare* wheat flour did not decrease loaf volume or affect mixing requirements, and crumb color was not greatly impaired. Durum flour increased absorption and loaf volume when blended with low protein English wheat flour. The effect of specific varieties of bread and durum wheat was not studied by these workers, and the present paper discusses this problem.

Materials and Methods

The flours were experimentally milled on the Allis-Chalmers mill from pure varieties grown at Langdon, North Dakota in 1950. All samples were sound and free from damage. Table I shows the varieties and their wheat characteristics.

The three hard red spring wheats are all widely grown in North Dakota, while for the durum, Mindum and Stewart are the more important in acreage. Nugget is probably the best in macaroni-making quality; Kubanka was one of the first of the durum varieties grown for macaroni-making, while 308 is a newer hybrid with good agronomic features but inferior in macaroni quality. These wheats cover a wide range in macaroni quality. All were exceptionally low in protein content because of weather conditions in 1950. The varieties are representative of those potentially useful.

The cleaned wheats were milled by the procedure described by Sibbitt, Scott, and Harris (7). One of the series of blended wheats was also milled without attempting to produce uniform low grade standard flour to discover what effect this procedure would exert on flour yields with varying proportions of durum wheat. The flours were baked by the micro malt-phosphate-bromate method (Geddes and Aitken, 3) with 5% sucrose. Methods of analysis for protein, ash, moisture, and diastatic activity have been described (1).

The durum flours were blended with the hard red spring and white wheat flours at the following concentrations: 10, 15, 20, 25, 50, and 75% by weight of the total flour in the blend.

Results

Milling. Table I gives miscellaneous data on the wheats and the flours which were experimentally milled from them. The test weights were all rather high, with Kubanka being the lowest. Vitreous kernel content was slightly higher for the hard red spring varieties. The protein content was lower than might be anticipated for all samples except Golden. Flour yields did not differ greatly, although Stewart, Carleton, and Golden were lowest. For ash, the bread wheat flours were markedly lower, as would be expected from their milling characteristics.

TABLE I

Protein Content, Milling, and Miscellaneous Data for the Wheat Varieties

(Arranged in order of decreasing total flour yield within class)

Variety	Test Weight	Grade[1]	Vitreous Kernels	Protein Content[2]		Flour Yield			Ash[2]
				Wheat	Flour	Long Patent	Low Grade	Total	
	lbs./bu.		%	%	%	%	%	%	%
Bread Wheats									
Rival	60.3	1 HDNS	90	12.5	11.6	71.4	3.0	74.4	0.43
Mida	62.1	1 HDNS	90	12.2	11.3	70.4	2.9	73.3	0.41
Cadet	60.6	1 HDNS	90	11.9	11.0	69.4	3.4	72.8	0.43
HRS average	61.0	—	—	12.2	11.3	70.3	3.1	73.5	0.42
Golden	59.2	2 HW	85	8.7	7.3	65.4	2.6	68.0	0.39
Durum Wheats									
Nugget	62.0	1 HAD	80	11.6	10.6	71.0	5.1	76.1	0.60
308	60.4	1 HAD	85	11.3	10.4	70.8	5.0	75.8	0.65
Kubanka	58.5	2 HAD	80	12.7	11.7	69.6	3.9	73.5	0.70
Mindum	60.3	1 HAD	80	11.7	10.7	69.3	3.1	72.4	0.65
Carleton	59.8	2 HAD	80	11.0	10.0	66.9	3.3	70.2	0.66
Stewart	60.1	1 HAD	80	11.2	9.8	66.0	3.6	69.6	0.67
Durum average	60.2	—	—	11.6	10.5	68.9	4.0	72.9	0.66

[1] HDNS = heavy dark northern spring.
HW = hard white.
HAD = hard amber durum.
[2] Expressed on 14.0% moisture basis.

TABLE II

Milling Data For Blends of Hard Red Spring and Durum Wheat

Blend	Flour Yield						Milling Time[3]
	Long Patent[2]	Ash[4]	Low Grade[2]	Long Patent[3]	Ash[4]	Low Grade[3]	
	%	%	%	%	%	%	*min.*
HRS[1] 100, durum 0	69.0	0.41	3.0	68.6	0.40	3.1	28
HRS 90, durum 10	68.9	0.43	3.3	69.1	0.42	3.4	29
HRS 85, durum 15	69.1	0.44	3.4	69.3	0.42	3.0	29
HRS 80, durum 20	67.5	0.42	4.0	69.7	0.46	3.0	31
HRS 75, durum 25	66.4	0.46	6.3	68.2	0.45	3.1	32
HRS 50, durum 50	66.7	0.51	5.3	68.5	0.53	3.1	34

[1] HRS = hard red spring.
[2] No milling deviations from regular flow sheet.
[3] Standard milling procedure, extra reductions if required to produce uniform low grade standard.
[4] Expressed on 14.0% moisture basis.

Table II shows the flour yields obtained from milling the hard red spring and durum wheat blends by the two procedures. For the regular millings, yield of long patent flour tended to decrease as the proportion of durum increased, with the result that the total flour yield remained approximately constant. When the blends were milled to a low grade flour standard, there was no consistent change in flour yield, but the milling time increased with durum wheat content.

TABLE III

BAKING DATA FROM THE ORIGINAL PATENT FLOURS
(Arranged in order of decreasing loaf volume within class)

Wheat Variety	Absorption	Loaf Volume	Grain and Texture[1]	Crumb Color[2]	Crust[3]	Symmetry[4]
	%	cc.				
Bread Wheats						
Mida	64.0	205	8.5	9.0	S	4.5
Cadet	66.8	200	8.0	8.5	S	4.5
Rival	66.4	195	8.0	7.5	S	4.5
HRS average	65.7	200	8.2	8.3	—	4.5
Golden	54.8	135	5.0 C&O	7.5	P	2.5
Durum Wheats						
Kubanka	58.0	145	6.0	5.5y	S	3.0
Carleton	57.2	125	5.0 C&O	4.5y	S	2.0
Stewart	57.2	125	4.0 C&O	5.0y	S	2.0
Mindum	57.6	120	5.0 C&O	5.0y	S	2.0
308	58.8	115	4.0 C&O	5.0y	S	1.5
Nugget	56.0	90	3.0 C&O	4.0y	D	1.0
Durum average	57.5	120	4.5	4.8y	—	1.9

[1] Grain and texture: C = coarse; O = open . . . . Perfect score 10.0.
[2] Durum loaves were demoted largely because of deep yellow color; little grayness apparent. . . . . Perfect score 10.0.
[3] Crust: S = satisfactory; D = dull; P = pale.
[4] Symmetry: all loaves were overoxidized . . . . Perfect score 5.0.

Baking Results from Original Flours. Table III shows the baking data from the original bread and durum wheat flours. Cadet and Rival had the highest absorption while Mida was significantly lower. Golden had the lowest absorption of all the wheats, with the durum varieties being intermediate between the hard red spring and Golden. There was little difference among the hard red spring wheat varieties in loaf properties, except crumb color, where Mida was much better than Rival. Golden was lowest of the bread wheats in all respects. The durums were markedly inferior to the springs in all factors except crust appearance, while Kubanka, the best among the durums, was

superior to Golden in loaf volume, grain and texture, crust and symmetry. Nugget was the poorest in all baking characteristics.

Absorption and Crumb Color. Figure 1 represents the effects of increasing amounts of durum wheat on the flour absorption and crumb color of bread and durum flour blends. For absorption of the hard red spring and durum flour blends, there was a decrease of approximately 1% following the addition of 10% of durum flour. At 15% of durum, the absorption decreased another 1% to 63.8%, the decrease

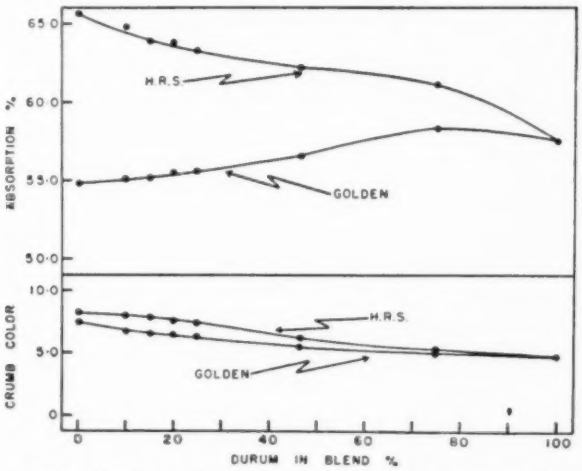

FIGURE 1. Effect on absorption and bread crumb color of increasing proportions of durum flour blended with hard red spring and Golden flours.

continuing to 57.5% for 100% durum flour. For Golden, the initial value of 54.8% increased to 55.1% for 10% durum flour, then became 58.3% at 75% of durum flour. Crumb color decreased gradually with both bread wheat flours as durum flour was added, with the spring wheat blends yielding generally the best results.

Loaf Volume. In Table IV are the loaf volumes of the blends for each wheat variety shown as the average obtained when baked with members of the other class. Loaf volumes of the unblended flours are included to facilitate comparisons. In the last column are average loaf volumes from all the blends for each variety. Little difference is evident among the hard red spring wheats in blending quality, but naturally Golden is much lower. The hard spring wheat flours can carry between 20 and 25% of the durum varieties with only a slight decrease in loaf volume and crumb color (Fig. 1). At 50% of durum flour, a significant decrease in loaf volume and crumb color occurred; at 75% of durum flour, these properties are still better than for the 100% durum flours.

TABLE IV

MEAN LOAF VOLUME OF THE ORIGINAL AND BLENDED VARIETIES
(Varieties arranged as in Table III)

Variety	Original Flour	Durum in Blends, %						Varietal Means, All Blends
		10	15	20	25	50	75	
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Mida	205	192	185	183	180	166	151	176
Cadet	200	197	192	187	186	170	159	181
Rival	195	192	191	187	182	172	151	179
Golden	135	137	136	135	132	132	131	134
Kubanka	145	181	181	181	179	175	165	177
Carleton	125	179	174	171	169	160	149	167
Stewart	125	177	175	171	170	162	146	167
Mindum	120	181	176	172	169	155	144	166
308	115	181	176	174	167	159	147	168
Nugget	90	176	172	170	166	149	132	161
Blend average	145	179	176	173	170	160	147	—

For the durums, Kubanka is the strongest and yielded the best loaves with all bread wheats. With Golden, Kubanka increased loaf volume from 135 cc. to a maximum of 165 cc. when the blend contained 75% of Kubanka flour. Apparently the mixing of the two wheat flours mutually improved their baking quality since the calculated loaf volumes for the 50% and 75% blends were 140 and 143 cc. respectively. This may be further evidence of the complementary effect of flour blends suggested by Merritt and Geddes (6). For the other durums there was little change in loaf volume with Golden, except for Nugget, where the 75% blend value was 110 cc.

LOAF VOLUME OF BLENDS OF FLOURS FROM KUBANKA AND NUGGET WITH GOLDEN

Durum in Blend, %								
0	10	15	20	25	50	75	100	Durum Variety
cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	
135	145	150	150	150	155	165	145	Kubanka
135	135	135	135	130	120	110	90	Nugget

Generally for the hard red spring wheat flours there was a gradual decline in loaf volume following the addition of durum wheat flours. No similar trend was apparent for the blends with Golden, where there was a decrease of only 4 cc. with 75% of durum flour. With Kubanka, the results were more favorable than for the other durum varieties as shown below. Clark and Martin (2) and Mangels (5) also found Kubanka best among durum varieties in baking quality. Since, as previously noted, the durum varieties were abnormally low in protein content, their effect on loaf volume in the blends with a weak flour was less than would normally be expected.

LOAF VOLUME OF BLENDS OF FLOURS FROM KUBANKA AND NUGGET WITH HARD RED SPRING

Durum in Blend, %								
0	10	15	20	25	50	75	100	Durum Variety
cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	
200	193	192	192	188	182	165	145	Kubanka
200	190	185	182	178	158	140	90	Nugget

Table V shows an analysis of variance of the loaf volume of the blended flours. As anticipated, the bread wheat varieties exerted the greatest effect on loaf volume, chiefly because of the poor baking quality of Golden. Differences in the proportions of hard red spring and durum wheat had the second greatest influence on loaf volume.

TABLE V

ANALYSIS OF VARIANCE OF LOAF VOLUME DATA

Source of Variation	Degrees of Freedom	Variance[1]
Bread varieties	3	18544.9
Durum varieties	5	650.7
Blends	5	3433.2
Interaction:		
Bread × durum varieties	15	131.2
Bread varieties × blends	15	269.8
Durum varieties × blends	25	59.6
Bread × durum varieties × blends	75	16.5
Total	143	

[1] All variances attained significance at the 1% point when tested by the appropriate error.

Among the interactions, the bread wheat varieties again had the greatest effect because of the poor strength of Golden. It is also apparent that the bread wheat varieties have a differential reaction with the durums, that is, all the bread wheat and durum varieties do not have the same effect on loaf volume as the proportion of durum flour is increased. Thus, variety has a significant effect on loaf volume for both bread and durum flours when they are blended in various proportions.

Diastatic Activity. Figure 2 provides information on the diastatic activity of the original, unblended flours. The durum flours are markedly higher in activity than the bread wheat flours, as would be expected, since durum wheats require more severe grinding during milling. The comparatively new variety, Nugget, is highest of all, while Kubanka is the lowest among the durums. The intermediate position of Kubanka corresponds with its baking quality. There are no marked differences between the other four durum varieties. For the bread wheat flours, Cadet and Rival are highest, but the difference between them and the other two varieties seems scarcely significant.

The effect on diastatic activity was determined by adding durum to hard red spring wheat and milling the blend by two methods. As might be expected the effect was additive, showing that the diastatic activity of bread wheat flours can be markedly increased by addition of durum in the mill mix. Although the durum flour milled by the regular method appeared to yield lower activity between 20% and 50% concentration, at 100% the results were identical.

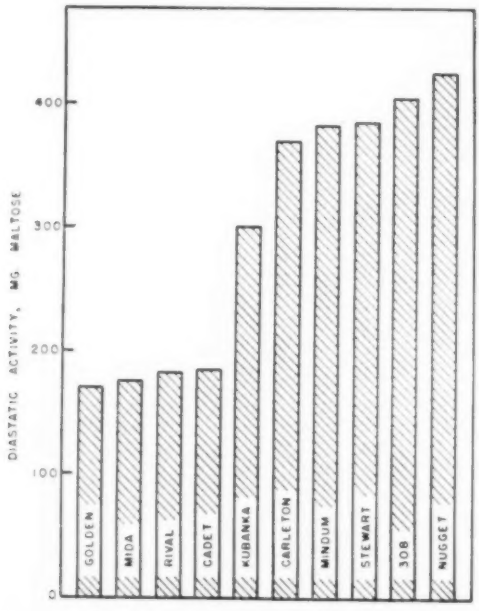

FIGURE 2. Diastatic activity of the original flours milled from hard red spring, Golden, and durum wheats.

Mixograms. Figure 3 shows the mixing requirements of some representative blends of hard red spring and durum wheat flours. The addition of durum flour did not materially affect the pattern until a 50% blend was attained. Further increase of durum flour caused a weakening of the mixogram, which approached the characteristics of a durum flour curve. Kubanka produced substantially the best curve among the durums, while Nugget was very poor, with little dough development.

Figure 4 similarly represents the mixogram patterns of the Golden and durum wheat flour blends. For the bread wheat flours, Golden produced the weakest mixogram, as would be expected. It was markedly inferior to the pattern yielded by Kubanka and slightly inferior to that of Stewart and possibly Nugget. As the proportion of durum

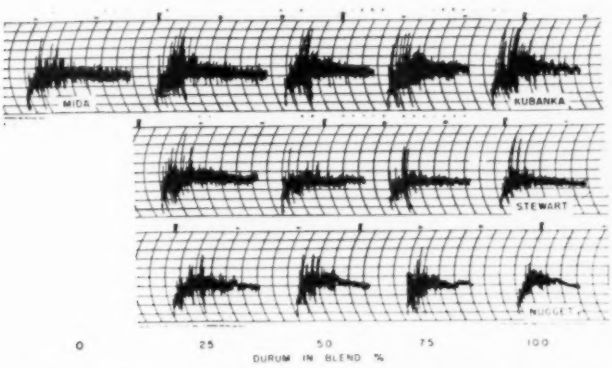

FIGURE 3. Effect on mixogram pattern of adding flour milled from different varieties of durum wheat to a representative hard red spring wheat flour.

increased, the strength of the curves also improved, with Kubanka exerting the greatest effect. As for the hard red spring wheats, the influence of the durum flour became evident at 50% of durum flour except for Kubanka, which strengthened the curve at 25% concentration.

Durum Flour Bleaching. Flours milled from Carleton and Kubanka were bleached with 0.14 g. of a commercial preparation of benzoyl peroxide, and matured with 0.12 g. of ammonium persulfate per 1 lb. of flour and blended immediately before baking at 10 and 25% concentrations with unbleached flours from Mida and Golden. Table VI shows the resultant data. For Carleton, bleaching and maturing did not improve loaf volume, but did slightly raise the grain and texture score, and considerably increased crumb color. For Kubanka, this

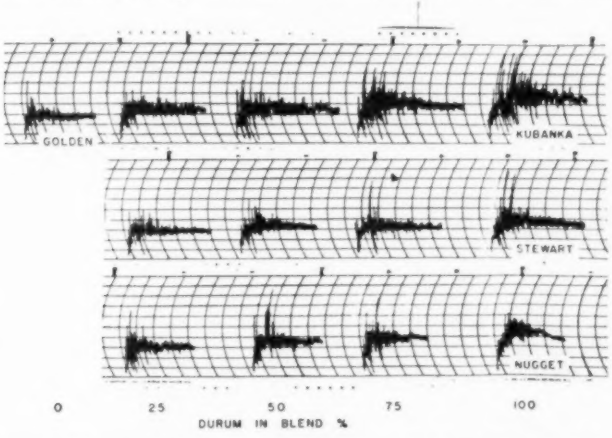

FIGURE 4. Effect on mixogram pattern of adding flour milled from different varieties of durum wheat to weak flour.

TABLE VI

EFFECT OF BLEACHING DURUM FLOUR ON THE BAKING PROPERTIES OF BREAD AND DURUM WHEAT FLOUR BLENDS[1]

Original or Blended Bread/Durum Flours	Absorption		Loaf Volume		Grain and Texture[2]		Crumb Color[3]	
	Un	Bl	Un	Bl	Un	Bl	Un	Bl
	%	%	cc.	cc.				
Mida, 100%	64.0	—	200	—	8.5	—	9.0	—
With Kubanka, 90/10	63.2	63.2	190	190	8.0	8.0	8.5	9.0
With Kubanka, 75/25	61.2	61.2	185	180	7.5	8.0	8.0	9.0
With Carleton, 90/10	63.2	63.2	190	195	8.0	8.5	8.0	9.0
With Carleton, 75/25	61.2	61.2	185	180	7.5	8.5	7.5	9.0
Golden, 100%	54.8	—	140	—	4.5 C&O	—	7.5	—
With Kubanka, 90/10	55.2	55.2	145	150	4.5 C&O	5.5 C&O	7.0	7.5
With Kubanka, 75/25	55.6	55.6	150	155	4.5 C&O	5.5 C&O	6.5	7.5
With Carleton, 90/10	55.2	55.2	130	135	4.5 C&O	4.5 C&O	7.0	7.5
With Carleton, 75/25	55.6	55.6	120	135	4.5 C&O	4.5 C&O	6.5	7.5
Kubanka, 100%	58.0	58.0	145	160	6.0	7.0	5.5y	8.5
Carleton, 100%	57.2	57.2	125	125	5.0 C&O	5.5 C&O	4.5y	6.5y
Averages (Without bread flours)	58.6	58.6	156	160	6.0	6.6	6.9	8.1

[1] Un = unbleached; Bl = bleached.
[2] Grain & texture; C = coarse, O = open.....Perfect score 10.0.
[3] Crumb color; y = yellow.....Perfect score 10.0.

treatment improved loaf volume, grain and texture, crumb color and symmetry. Bleaching increased the blend loaf volume little or none, but improved crumb color significantly and grain and texture slightly. There was no effect on absorption.

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MILL SANITATION STUDIES. I. RELATIVE SUSCEPTIBILITIES OF MILL STOCKS TO INFESTATION BY THE CONFUSED FLOUR BEETLE[1]

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ABSTRACT

Assessments were made of the relative susceptibilities of three grades of each of four principal mill stocks to *Tribolium confusum* on the basis of the criteria: mean time for larval development; survival of larvae; number of progeny produced; mean time to produce progeny; and survival of adults. Combinations of these criteria in the form of Stanley's environmental index facilitated comparisons of the abilities of the stocks to support *T. confusum*.

The productivity of *T. confusum* was significantly greater on tailings and low-grade flours than on middlings or break stocks. However, adult survival during 6 months was high on middlings and break stocks, and low on tailings and low-grade flours. The productivity of *T. confusum* was different on different grades of the same stock except on tailings stocks, all grades of which favored high productivity. Tailings stocks and 3rd low-grade flours supported the production and development of the insect at about four times the rate of production on 1st-break stock, and three times the rate on 2nd middlings. No simple relationship is apparent between the productivity of *T. confusum* and the chemical and physical composition of mill stocks.

In many types of milling machines, small quantities of the stock passing through the machines become lodged and available for the establishment of insects. The conditions of temperature and humidity in these machines are generally close to the optimum for insect pests of cereal products; hence, if the lodged stock is a favorable food, established insects will multiply rapidly and form a nucleus of infestation from which insects may spread to other parts of the milling system. Mill stocks differ widely in their nutrient and physical compositions, and the confused flour beetle, *Tribolium confusum* Duv., a common mill insect, is known to be sensitive to such differences (1, 3). Accordingly, some mill stocks would be expected to favor the development of insect infestation more than others. Knowledge of the productivity, devel-

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opment, and survival of insects on a variety of mill stocks would enable millers to anticipate where infestations are most likely to become established, and to concentrate control measures at such places.

This study was designed to evaluate the relative susceptibilities of principal mill stocks to *T. confusum*. The criteria used to assess susceptibility were the productivity, development and survival of the insects; initial preference of the insects for the mill stocks was not studied. Therefore, the study yields information on the ability of the various mill stocks to support the reproduction and development of insects which became established in them by choice or by chance.

Materials and Methods

Three grades of each of four mill stocks were selected.[4] The common mill designation and the analysis for each grade are shown in Table I. Samples of each grade were taken from regular mill streams, and 125-ml. aliquots of these samples contained in half-pint "Sealrite" cartons were used for all tests. Before introduction of the insects, the stocks were conditioned for 7 days at 27°C. and approximately 75% relative humidity, and all tests were conducted at this temperature and humidity.

TABLE I

Designation and Analysis of Mill Stocks

Stock Designation	Protein	Ash	Fibre	Fat	Per Cent of Stock Retained (+) or Through (−) Sizing Screens			
					+24W	+68	+10XX	−10XX
	%	%	%	%				
1st Break	14.3	1.92	2.86	2.44	86.0	7.5	2.5	4.0
3rd Break	15.2	2.80	4.68	3.28	54.0	15.0	11.0	20.0
4th Break	15.8	4.52	8.36	4.70	67.0	15.0	3.5	14.5
2nd Middlings	11.3	0.41	0.20	0.96	0.0	9.0	48.0	43.0
3rd Middlings	11.9	0.49	0.28	1.03	0.0	7.0	46.5	46.5
5th Middlings	11.9	0.56	0.32	1.44	0.0	2.5	42.0	55.5
1st Low-Grade	11.5	0.57	0.32	1.03	0.0	1.0	26.0	73.0
2nd Low-Grade	13.4	0.81	0.50	1.74	0.0	6.5	31.5	62.0
3rd Low-Grade	12.8	1.00	0.79	1.58	0.0	16.0	42.5	41.5
Germ Tailings	15.4	2.90	3.35	4.88	46.5	30.5	8.5	14.5
1st Tailings	12.8	1.82	2.12	3.04	5.0	46.0	18.0	31.0
4th Tailings	12.8	1.20	1.03	1.82	0.0	20.5	49.0	30.5

The susceptibilities of the various stocks were assessed on the basis of the following criteria: mean time for larval development; survival of larvae; number of progeny produced; mean time to produce progeny; survival of adults. Duplicate determinations were made for each criterion on each of the 12 mill stocks.

[4] Courtesy of the Ogilvie Flour Mills, Winnipeg, Canada.

Larval development and survival were determined by rearing 50 larvae, 0–1 days old, on duplicate samples of each stock. Seventeen days after the larvae were placed on the stocks, daily examinations for pupae were begun, and these were continued until all surviving larvae had pupated. The number of pupae recovered at each examination was recorded, and the pupae were removed from the stock. The total number of pupae recovered yielded the data on larval survival, and the daily record of larvae reaching pupation provided the data for calculation of the mean time for larval development.

The number of progeny produced was determined by introducing 25 males and 25 females, 0–5 days old, into duplicate samples of each stock for a conditioning period of 14 days and then transferring them to fresh samples of the same stock, where they remained undisturbed for 30 days. The adults were then removed. Progeny resulting from reproduction of the adults during the 30-day period were recovered as they reached the pupal stage; recovery at any earlier stage was impossible in the coarser stocks. The pupae were counted and removed from the stock at 4-day intervals. The total number of pupae recovered from each stock gave the number of progeny produced; the mean time to produce the progeny was calculated on the basis of the frequency distribution for pupae recovered and time between introduction of the adults into the stock and removal of the pupae.

Survival of adults was determined over a period of 6 months. Fifty young adults, 0–4 days old, were introduced into duplicate samples of each stock. At 30-day intervals the surviving adults were transferred to fresh stock so that the progeny were never allowed to develop to adults in the presence of the original beetles. Mortality was determined at weekly intervals during the 6-month period.

All data were subjected to analysis of variance and are reported as means, with the necessary differences for significance.

Results and Discussion

In general, the insects showed greater sensitivity to differences between the principal mill stocks (breaks, middlings, etc.) than between grades of the same stock (1st break, 3rd break, etc.). The lumped data for the three grades of each stock are considered in the following section, and the data for individual grades of each stock are dealt with in a second section.

Differences Between Stocks. Table II shows the development and survival of *Tribolium confusum* larvae on the four stocks. The rate of larval development was affected by the type of stock on which the larvae were reared. Development was most rapid on tailings and slowest on break stocks, and the difference is significant; Gray (3)

obtained similar results for the development of *T. confusum* on long-extraction flours and whole-wheat meal. Table II shows that the survival of larvae on the four types of stock was very uniform, and on this criterion the stocks were indistinguishable.

TABLE II

MEAN DATA FOR EFFECTS OF MILL STOCKS ON *T. confusum* LARVAE

Stocks	Development	Survival	Environmental Index[1]	
			Relative	Absolute
	days	*%*		
Breaks	22.8	94.3	4.14	0.95
Middlings	22.3	94.3	4.23	0.97
Low-grade flours	22.1	95.3	4.31	0.99
Tailings	21.8	95.0	4.35	1.00
Least significant difference, 5% level	0.6	3.2	0.17	

[1] See text.

Miller (5) found that tailings stocks favored more rapid development of larvae than white flours of low extraction (patent flours); 1st-break stock was only slightly less favorable than tailings. Table II shows that at 27°C. larvae developed more slowly on breaks than on any other stocks. At 30°C. larvae develop more rapidly (3). Rapid larval development is associated to some extent with high moisture content (4). Products milled from Canadian hard spring wheat have higher equilibrium moisture contents than those derived from Australian soft wheat and would therefore favor more rapid larval development. In general, Miller's results with tailings stocks are comparable to those shown in Table II. However, it must be remembered that, due to various factors, the results of the two studies on larval development cannot be too closely compared. These factors include differences in moisture content and milling characteristics of the two types of wheat, and differences in the milling systems used in Canada and Australia.

It is evident from Table II that tailings favor the rapid development of the insect but have no significant effect upon the survival. Interpretation of these results presents a difficulty that becomes apparent whenever two or more biological criteria are used to assess the effect of varying some environmental factor. For instance, high temperatures favor rapid development of *T. confusum* but this rapidity is accompanied by low survival; at lower temperatures, slow development is associated with high survival (7). The difficulty is to express the total response of the organism in terms of measurable functions,

such as rate of development and survival, which have no necessary relationship.

Stanley (7) derived a ratio, the environmental index, based on observations of the survival and rate of development of *T. confusum*, and used this ratio to assess the suitability of a range of temperatures. His basic concept is that the environment most suitable to an animal is that in which it "accomplishes the most per unit time in furthering the increase of the species." He derived a relative environmental index, E_r, equivalent to S/T, S being the percentage survival during a given developmental stage and T the mean time required to complete this development. E_a is the absolute environmental index, obtained by expressing the values of E_r as decimal fractions of the optimum value

TABLE III

MEAN DATA FOR EFFECTS OF MILL STOCKS ON *T. confusum* ADULTS

Stocks	Mortality During 6 Months	No. Progeny Produced[1]	Mean Time to Produce Progeny [1]	Environmental Index for Production	
				Relative	Absolute
	%		*days*		
Breaks	29.0	562	45.9	12.5	0.50
Middlings	27.0	645	46.1	14.2	0.57
Low-grade flours	41.0	963	43.0	22.4	0.90
Tailings	43.0	1045	42.1	24.8	1.00
Least significant difference, 5% level	9.5	189	2.7	4.6	

[1] As pupae resulting from reproduction of 50 adults during 30 days.

of E_r. The values of E_a will then vary from zero, under conditions where the insect cannot exist, to one under optimum conditions. However, a truly optimum value of E_r under all conceivable conditions is not available, and the highest value of E_r obtained in a series of observations is used for the calculation of E_a. Therefore, E_a derived in this way is not an absolute index but provides a convenient scale for comparing the values of E_r. A "growth index," identical in form to Stanley's "relative environmental index," was used by Trager and Subbarow (8) to assess the nutritional requirements of mosquito larvae.

Table II shows the relative and absolute environmental indexes for *T. confusum* larvae reared on the four mill stocks. The values of the relative environmental index indicate that the number of larvae completing development per unit of time was greatest on tailings and least on break stocks. This follows from the more rapid rate of development on tailings and the uniform survival on all stocks. Apart from this difference, the index shows that the stocks supported the development and survival of the larvae about equally.

The stocks were more clearly differentiated by their effects on adults of *T. confusum* as shown in Table III. On the basis of the mortality of adults during a 6-month period the stocks are divided into two groups: breaks and middlings, on which the insects showed low mortalities; and low-grade flours and tailings, which promoted relatively high mortalities. Pearl, Park and Miner (6) found the mean life span for *T. confusum* to be 188 days when the insect was fed on yeast-enriched flour. On breaks and middlings the mean mortality was 28% at 180 days, indicating that these stocks markedly favor survival.

On the basis of the number of progeny produced and matured to the pupal stage (Table III) the stocks are divided into the same two groups, significantly fewer progeny being produced on breaks and on middlings than on low-grade flours or tailings. Hence, there seems to be a relation between the number of progeny produced and the survival of adults, low survival being associated with high reproductive activity and vice versa. Fraenkel (2) suggested that the survival of *T. confusum* larvae on an incomplete diet depends on metabolic activity.

The stocks were differentiated into the same two groups on the basis of the mean time required to produce and mature progeny to the pupal stage (Table III). Pupae were produced more slowly on breaks and on middlings than on low-grade flours or tailings. This difference must have resulted from a difference in the rate of larval development on the two groups of stocks. Such a difference in rate of development was found when larvae alone were reared on the four mill stocks (Table II).

Table III shows the environmental index for production calculated as the ratio of the number of progeny produced and the time required to produce them. This ratio provides a comprehensive index of the suitability of the mill stocks for *T. confusum* because it takes into account the number of eggs laid, the mortality of eggs and of larvae, and the rate of larval development. Its prognostic value is also enhanced because it includes the effects of the normal associations and interactions of the various life-history stages, for instance, egg-eating by adults.

The values for the relative environmental index shown in Table III enable quantitative comparison of the productivity of *T. confusum* on the four mill stocks. On the basis of the index the stocks are divided into two groups: breaks and middlings, on which the rate of production and development of progeny was low; and low-grade flours and tailings, on which these rates were high. The values for the "absolute" index show that, on tailings, progeny were produced and matured at twice the rate of production on breaks; low-grade flours were markedly more

favorable for the rapid increase of *T. confusum* than were middlings. These results confirm the general observation by millers that "germy" stocks are most susceptible to insect infestation.

Differences Between Grades of Stocks. Differences between grades of the four mill stocks were demonstrated only in production of progeny. Table IV shows the numbers of progeny that developed to the pupal

TABLE IV

MEAN DATA FOR PRODUCTION OF *T. confusum* ON GRADES OF MILL STOCKS

Grades of Stocks	No. Progeny Produced [1]	Mean Time to Produce Progeny [1]	Environmental Index	
			Relative	Absolute
		days		
Breaks				
1st	305	48.9	6.3	0.24
3rd	714	44.9	15.9	0.59
4th	666	44.0	15.2	0.57
L.S.D., 5% level	199	3.3	4.9	
Middlings				
2nd	460	49.4	9.3	0.35
3rd	642	44.2	14.5	0.54
5th	832	44.6	18.6	0.69
L.S.D., 5% level	122	8.6	2.5	
Low-Grade Flours				
1st	840	43.8	19.2	0.72
2nd	917	42.8	21.4	0.80
3rd	1134	42.4	26.7	1.00
L.S.D., 5% level	134	3.7	3.7	
Tailings				
Germ	961	41.2	23.2	0.87
1st	1093	42.5	25.7	0.96
4th	1081	42.6	25.4	0.95
L.S.D., 5% level	261	5.3	4.7	

[1] As pupae resulting from reproduction of 50 adults during 30 days.

stage, and the mean times to produce them, on three grades of each of the four mill stocks. On break stocks, the values for the relative environmental index show that the 1st break was significantly unfavorable as compared with the 3rd and 4th breaks. The apparent low susceptibility of break stocks, particularly the 1st break, may be related to unequal distribution of nutrients in the fractions of different particle size and the preferential feeding by insects on certain of these fractions. For instance, in the 1st-break stock, the finer particles are probably mostly endosperm low in essential nutrients. On middlings,

the index shows that the insects' productivity increased progressively from the 2nd to 3rd to 5th grades. Low-grade flours supported a generally high level of productivity, with the 3rd low-grade significantly more favorable than the other two. The insects were equally productive on the three grades of tailings stock, and these grades supported the highest mean level of productivity.

The "absolute" environmental index is based on the optimum value of the relative index, namely, the value for 3rd low-grade flour; however, the values for 1st and 4th tailings are not significantly different. Hence, these three grades are indicated as the most susceptible of the grades investigated, and as capable of supporting the production and development of progeny at about four times the rate on 1st-break stock and three times the rate on 2nd middlings.

Comparison of these results with the analysis of stocks shown in Table I indicates that within stocks the productivity of *T. confusum* on the various grades varies generally with the fat and fibre content. This is most apparent in the three grades of middlings, which show an increase in fat and fibre content from the 2nd to the 5th grade and a corresponding increase in productivity. The same association is apparent in the breaks and the low-grade flours, but not in the tailings. If, however, the 12 grades are considered without reference to the stocks from which they are derived, the relation between fat and fibre content and insect productivity does not hold. For instance, low insect productivity on the 1st break is associated with a higher fat and fibre content than the analysis shows for the 3rd low-grade flour, on which *T. confusum* was most productive. Factors additional to the fat and fibre content, with the associated vitamins of the B complex, must determine the ability of mill stocks to support the production and development of *T. confusum*. As indicated by Gray (3) one such factor may be the particle size of the stock; Table I shows that the grades containing higher proportions of particles passing through a 10XX screen were more favorable for *T. confusum* on break stocks, middlings, and tailings stocks; the reverse was true for low-grade flours. The interaction of particle size and chemical composition of mill stocks may be involved in the observed differences in productivity of *T. confusum* on different mill stocks.

Under mill conditions, the population of insects in various mill stocks will be a function of the number of insects attracted to the stocks as well as the favorability of the stocks for reproduction and development. Recent work by Willis and Roth (9, 10) has shown that the attraction of *T. castaneum* to flour is affected by the moisture content of the flour; depending on whether the insects are starved or unstarved they may be attracted or repelled by food with a high moisture content.

However, the milling process is such that differences in the moisture content of the various mill fractions are small, and it seems unlikely that moisture content would have much effect on the distribution of insects between stocks. Under practical conditions, susceptibility of a mill stock will depend not only on the productivity of insects established in the stock but also on the members introduced into it; the factors determining initial distribution and introduction are not known but two possibilities exist, by chance and by choice (attraction). The finding of Willis and Roth, namely, that selection is governed by moisture content does not appear applicable here because in their study response was conditioned by starvation. On the other hand, continuous movement of stocks in mills and random movement of beetles suggest distribution by chance. On this assumption, the present findings on productivity of insects in stocks afford reasonable prediction of the distribution of numbers of insects between stocks in mills.

From the standpoint of practical insect control in flour mills, this study indicates that tailings stocks and the rougher grades of low-grade flours are most likely to promote the rapid development of *T. confusum* populations. After a general fumigation, attention to machines carrying these stocks should not be relaxed, for they are most likely to become the nuclei of fresh outbreaks. The finer grades of low-grade flours and the grades of middlings arising toward the end of the milling process (4th middlings and later) are scarcely less suspect. Break stocks and the finer grades of middlings appear least likely to initiate and foster infestation. Unfortunately, these stocks and their derivatives demand the miller's greatest effort in preventing insect contamination of finished products.

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THE EFFECT OF VARIOUS MILK SERUM PROTEINS AND THEIR SULFHYDRYL GROUPS ON BREAD QUALITY [1]

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ABSTRACT

Baking studies with purified milk serum protein fractions have revealed that β-lactoglobulin, which can account quantitatively for most, if not all, of the sulfhydryl reducing capacity of milk and which makes up 50 to 60% of the total serum proteins, is not deleterious to dough consistency and loaf volume when incorporated into doughs. Furthermore, bovine blood serum albumin and colostrum immune euglobulin and pseudoglobulin, each of which comprise about 5% of the total serum proteins of milk, are not deleterious. Heat treatment of the serum proteins, and of β-lactoglobulin, results in an activation of the very unreactive sulfhydryl groups present in the native protein and these do have a somewhat deleterious action on baking quality. Egg albumin behaves similarly to β-lactoglobulin.

It appears that the sulfhydryl groups of milk proteins are not the factor responsible for the poor baking quality of milk which has not received an adequate heat treatment. Consequently any method for measuring them would not necessarily measure baking quality. These findings imply that all of the methods which are based on the measurement of the reducing capacity-or the amount of undenatured serum proteins are fundamentally unsound as absolute indices of the baking quality of nonfat dry milk solids since they measure materials and properties which in themselves are not deleterious.

Extensive work (4, 7) has shown that the serum protein fraction of milk is responsible in part for the deleterious action on dough and bread of insufficiently heated milk or nonfat dry milk solids derived therefrom. Since the work of Stamberg and Bailey (17), it has been thought that the beneficial effect of heat treatment on the baking quality of skim milk is due to the removal or alteration of sulfhydryl groups present. Larsen *et al.* (8) demonstrated that the total sulfhy-

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dryl content of the milk proteins, as measured by an *o*-iodosobenzoate-iodine titration procedure, decreased upon heat treatment and they postulated that the sulfhydryl groups were rendered sterically unavailable by the heat treatment and hence were no longer deleterious to dough quality. More recently Larson and Jenness (9, 10, 11) have shown that this decrease in *o*-iodosobenzoate-iodine titer is actually due to oxidation of the sulfhydryl groups by molecular oxygen. It was further demonstrated that the sulfhydryl groups of unheated milk are very unreactive but that those remaining unoxidized in heated milk are very reactive. A similar increase occurs in the reactivity of the sulfhydryl groups of egg albumin upon heat denaturation.

The serum proteins of milk consist of a number of fractions. β-Lactoglobulin, a crystallizable protein, comprises a considerable portion of the mixture (16). In fact, electrophoretic analysis indicates that proteins with the mobility of β-lactoglobulin represent 50 to 60% of the total serum proteins although yields of the crystalline product have never been obtained in such a proportion. Polis *et al.* (13) have recently shown by electrophoretic and isolation procedures that a fraction apparently identical with normal bovine blood serum albumin constitutes about 5% of the milk serum proteins. Smith (15, 16) described the preparation from milk serum of a euglobulin and a pseudoglobulin which together represent 10 to 20% of the serum proteins and both of which are involved in immunological reactions. These last two proteins are present in much higher concentration in colostrum than in normal milk.

Probably β-lactoglobulin is the principal sulfhydryl protein of milk serum. Larson and Jenness (11) calculated that if the fraction which has the electrophoretic mobility of β-lactoglobulin had the sulfhydryl titer of β-lactoglobulin, it alone would account almost exactly for the entire sulfhydryl titer of the serum proteins and of milk itself. Thus, β-lactoglobulin is the major, if not the only, component measured by various methods which have been proposed to determine the baking quality of nonfat dry milk solids by measurement of protein reducing capacity and it is the major component measured by various methods for determining the amount of serum protein present (3, 12, 14). Bovine serum albumin has a low content of either active or potentially active sulfhydryl groups (2, 5). The sulfhydryl content of the other milk proteins is not known.

With these considerations in mind, it appeared desirable to compare the effects of crystalline β-lactoglobulin and of other purified milk serum protein fractions when incorporated into bread doughs. In addition, crystalline egg albumin was included in the study since the activity of the sulfhydryl groups of this protein has been characterized by many investigators.

Materials and Methods

Crystalline β-lactoglobulin was prepared essentially according to the method of Bull and Currie (1) and crystalline egg albumin by the method of Kekwick and Cannan (6); both proteins were recrystallized four times. The immune euglobulin and pseudoglobulin fractions were isolated from a mixed sample of the colostral milk from two cows according to the procedure of Smith (15). The bovine blood serum albumin was a commercial crystalline product (Armour). The milk serum proteins were prepared by precipitating the casein from raw skim milk using 100 ml. of 10% acetic acid and 100 ml. of 1 *N* sodium acetate per liter of milk, exhaustively dialyzing the filtrate against distilled water, and freeze-drying. All of the milk protein preparations, with the exception of the serum protein preparation, were electrophoretically homogeneous.

Two series of experiments were made. In the first, egg albumin, β-lactoglobulin, and the serum proteins were dissolved in phosphate buffer (pH 6.9, $\mu = 0.1$) and subjected before baking to the following treatments to alter their sulfhydryl group activity (9):

(1) None. These samples of the native proteins possess the total sulfhydryl content, but the groups are very unreactive.

(2) Heated for 30 minutes at 78°C., and added to dough immediately after the heat treatment. This treatment gives the maximum of very reactive sulfhydryl groups which are "liberated" during the heat treatment and before those of β-lactoglobulin are further oxidized by molecular oxygen.

(3) Heated for 30 minutes at 78°C., and stored at 4°C., for 72 hours before baking. This treatment gives an opportunity for the very reactive sulfhydryl groups to be oxidized.

(4) Heated for 30 minutes at 78°C., treated with 0.5 g. of iodoacetamide per 75 ml. of solution and exhaustively dialyzed against phosphate buffer for four days. Iodoacetamide will combine with most of the sulfhydryl groups of denatured egg albumin and most of those present in β-lactoglobulin which are not attacked by molecular oxygen. Since iodoacetamide itself is a flour improver, acting like bromate, exhaustive dialysis was necessary to remove all of it. This treatment should remove all of the very reactive, as well as the mildly reactive, sulfhydryl groups of the three protein systems.

(5) Treated with acid and potassium iodide as in the regular *o*-iodosobenzoate-iodine titration procedure (10), *o*-iodosobenzoate added until a slight excess was present, sodium hydroxide added to bring the pH to 6.9, and the resultant system exhaustively dialyzed against phosphate buffer for four days. This treatment was applied to β-lacto-

globulin only. The resultant protein sol was water-clear like that of native β-lactoglobulin but had no reducing titer whatever.

(6) Treated as in (5) except that the sample was heated for 30 minutes at 78°C. before the oxidation. This treatment was applied to β-lactoglobulin only.

In the second experimental series, the serum proteins, β-lactoglobulin, euglobulin, pseudoglobulin, blood serum albumin, and a mixture of the latter four proteins in the ratio of 10:2:2:1 were dissolved in phosphate buffer (pH 6.9, $\mu = 0.1$). These solutions were given the same treatments as in the first three steps of the first series, i.e., unheated, heated immediately before baking, and heated and held (in this case for 48 hours) before baking. In this second series, the sulfhydryl contents of the several protein sols were determined by the *o*-iodosobenzoate-iodine titration with and without the addition of iodoacetamide (9). The *o*-iodosobenzoate-iodine titration measures the total sulfhydryl groups and the difference between the titers with and without iodoacetamide is a measure of the "active groups."

In both series all of the protein preparations were incorporated into doughs at a level which would be present in a formula containing 6% nonfat dry milk solids (flour basis). Since 12 g. of nonfat dry milk solids contain about 1.0 g. of serum proteins of which 50 to 60% probably is β-lactoglobulin, respective levels of 1.0 g. and 0.5 g. per 200 g. of flour were employed for these proteins respectively. Egg albumin, which has a sulfhydryl content very similar to that of β-lactoglobulin, was incorporated at a level of 0.5 per 200 g. of flour. Bovine serum albumin, euglobulin, and pseudoglobulin, each of which comprises 5 to 10% of the serum proteins of milk, were incorporated at levels of 0.05, 0.10, and 0.10 g. per 200 g. of flour respectively. The required quantity of protein sample was made up in 75 ml. of phosphate buffer for each mix of 200 g. of flour.

The baking test was that of Larson *et al.* (12); the same brand of hard red spring wheat flour was employed. The bromate level was 1 mg. per 100 g. of flour. A constant absorption (64% in the first series and 63% in the second) was used for the water and buffer controls and for the doughs to which the protein sols were added. Control doughs containing "high-heat" (190°F. for 30 min.) and "low-heat" (145°F. for 30 minutes) spray dried nonfat milk solids at a 6% level (flour basis) were also included in the first series; an absorption was used for these which was found to yield the desired dough consistency. Two 150 g. portions were scaled off from each mix (200 g. flour) and both were given a 2-hour fermentation.

Results

The handling properties of the doughs during the baking procedure were practically identical and reflected no characteristic differences due to any of the protein fractions. The bread baked in both series was judged for quality characteristics and the loaf volumes were measured. The scores for the loaves were practically identical; only the loaf volume reflected differences.

The loaf volumes obtained in the first series are shown in Fig. 1. Native β-lactoglobulin and egg albumin did not lower the loaf volume. Upon heating, however, these two proteins caused a depression of the loaf volume. It seems logical to conclude that the unreactive sulfhy-

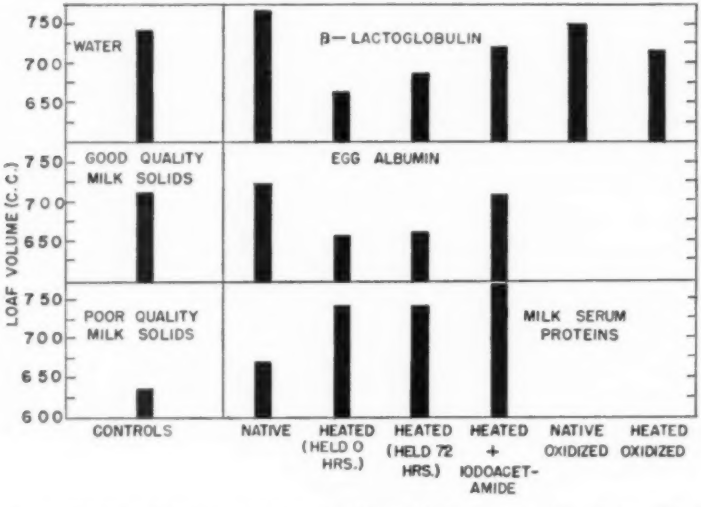

FIG. 1. The effect of the milk serum proteins, β-lactoglobulin, egg albumin, and skim milk given various treatments to change the sulfhydryl group activity on doughs containing them at a level equivalent to 6% nonfat dry milk solids (flour basis).

dryl groups of these two native proteins (1.30% cysteine for β-lactoglobulin and 1.11% for egg albumin (10)) have no relation to baking quality, but that the very reactive groups formed as a result of heating are deleterious to some degree. This explanation is supported by the findings that the oxidation of the reactive sulfhydryl groups or alkylation with iodoacetamide caused an increase in volume over that found with the freshly heated samples.

The intact serum protein mixture presents a completely different picture. In agreement with previous work (4, 7), the unheated serum proteins depressed loaf volume but this effect was overcome by heating. The loaf volume is still further improved by blocking the sulfhydryl

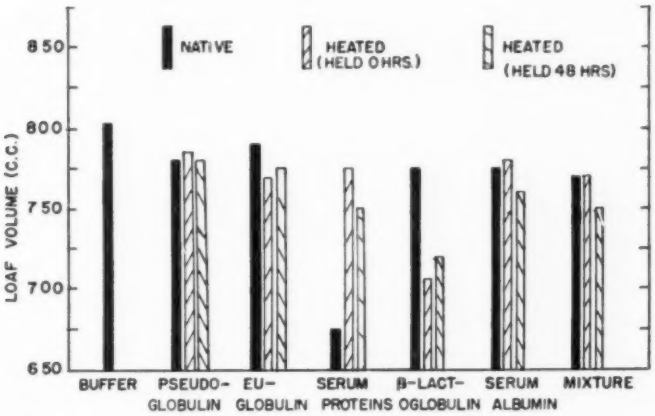

FIG. 2. The effect of various purified milk serum protein fractions given various treatments to change the sulfhydryl group activity on doughs containing them at a level equivalent to 6% nonfat dry milk solids (flour basis).

groups following the heat treatment, this effect being similar to that noted with purified β-lactoglobulin and egg albumin.

The loaf volumes obtained in the second series are shown in Fig. 2. In this series, unlike the first, sulfhydryl titrations were performed on aliquots of the solutions at the time of baking; these titers are presented in Table I. The results confirm the conclusions drawn from the first series relative to the effects of β-lactoglobulin and the serum proteins on loaf volume. The sulfhydryl results were as anticipated from previous

TABLE I

THE REDUCING POWER OF VARIOUS MILK PROTEINS BEFORE AND AFTER HEAT TREATMENT

Protein [1]	Solution Concentration	Protein Reducing Power					
		No Treatment		Freshly Heated [2]		Heated [2] and Held	
		Total [3]	Active [4]	Total [3]	Active [4]	Total [3]	Active [4]
	%	As mg. cysteine per 200 g.					
Pseudoglobulin	0.13	0.0	0.0	0.0	0.0	0.0	0.0
Euglobulin	0.13	0.0	0.0	0.0	0.0	0.0	0.0
Serum albumin	0.07	0.0	0.0	0.0	0.0	0.0	0.0
β-lactoglobulin	0.69	6.7	0.0	3.3	1.9	1.5	0.0
Serum Proteins	1.06	6.6	0.0	2.5	1.4	1.2	0.0

[1] All proteins in phosphate buffer, pH 6.9, $\mu = 0.1$.
[2] Heat treatment of 78° C. for 30 min. in air.
[3] Determined by an *o*-iodosobenzoate-iodine titer at the time of baking.
[4] Determined by adding 0.25 g. of iodoacetamide to 25 ml. of the solution at the time of baking and after two hours titrating by the *o*-iodosobenzoate-iodine procedure. The difference between this and the total titer (without the iodoacetamide present) is the "active" sulfhydryl group titer.

work with the serum proteins (11). Euglobulin, pseudoglobulin, and serum albumin, or a mixture of these three proteins with β-lactoglobulin, were not deleterious to baking quality.

Discussion

The results of these baking experiments indicate that β-lactoglobulin, the chief component of the serum proteins and responsible for the majority, if not all, of the sulfhydryl reducing titer of the serum proteins, is not deleterious to baking quality when incorporated into doughs. The effect of sulfhydryl groups on the baking quality is not observed in the native proteins but is only apparent after the proteins have been heated. The effect of the reactive sulfhydryl groups may be overcome by using appropriate methods to decrease the sulfhydryl group activity. Therefore, one is led to the conclusion that sulfhydryl groups do not apparently play a significant role in the effect of these native proteins in breadmaking. They do have an effect although relatively minor, when the proteins have been heated resulting in the presence of reactive sulfhydryl groups. Thus the deleterious effect of the unheated serum proteins, commonly attributed to sulfhydryl groups, apparently is due to an unknown action of some component or components of the serum proteins other than β-lactoglobulin.[4]

It is difficult to explain the fact that the freshly-heated serum protein mixture produced a greater loaf volume than the freshly-heated β-lactoglobulin in both series. If the deleterious effect of the serum proteins is due to a heat labile factor, and if the heated β-lactoglobulin reduced loaf volume through an action of its highly reactive sulfhydryl groups, it would be expected that heat treatment of serum proteins would increase the loaf volume to the level exhibited by freshly-heated β-lactoglobulin but not higher. To be sure, the level of active sulfhydryl groups was somewhat lower for the freshly-heated serum proteins than for the similarly treated β-lactoglobulin in one experiment (Table 1) but it is not considered that the difference is great enough to explain the differences in loaf volume. The explanation of this apparent discrepancy must await isolation of the factor or factors responsible for the deleterious action of unheated serum proteins.

β-Lactoglobulin, euglobulin, pseudoglobulin, and serum albumin together constitute up to 80% of the normal milk serum proteins and none of these are deleterious to baking quality at the level normally found in milk. Admittedly, the serum albumin was isolated from blood and the euglobulin and pseudoglobulin were isolated from colostrum, but there is considerable evidence that these proteins exist in the

[4] The possibility might be suggested that β-lactoglobulin does not depress loaf volume because it is altered by the ammonium sulfate treatment employed in its isolation. Such a contention is not valid, however, because serum protein precipitated at 80% saturation with ammonium sulfate still retains its ability to depress loaf volume. (Unpublished experiments).

same state in normal milk (13, 15). These findings may well explain why none of the methods devised to evaluate the amount of serum proteins present or the reducing capacity of milk have proved to be completely reliable in predicting the baking quality of an unknown sample of nonfat dry milk solids (12). It should not be overlooked, however, that these methods do reflect to a certain degree the amount of heat treatment the milk has received (12) and this heat treatment apparently determines the extent of "denaturation" of the deleterious factor or factors present in the milk serum proteins.

Acknowledgments

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LIPID BINDING IN DOUGHS. EFFECTS OF DOUGH INGREDIENTS[1,2]

D. K. MECHAM and N. E. WEINSTEIN

ABSTRACT

Much of the ether-extractable lipid of flours becomes "bound" when the flours are wetted or doughed (Olcott and Mecham, 9). These earlier observations were largely restricted to flour-water doughs; the effects of some other bread dough ingredients at levels in the range used in baking have now been determined.

Salt decreases lipid binding in doughs, both of total lipid and of phospholipid, to 20 to 40% less than that occurring in the absence of salt. A "softener" of the polyoxyethylene stearate type had somewhat similar effects. Shortening (lard) appeared to decrease phospholipid binding slightly, but did not affect total lipid binding appreciably. Other bread ingredients had no detectable effects.

The lipid contents of glutens washed out in salt solutions were lower than those washed out in water, paralleling the observations on lipid binding in doughs.

The suggestion was made by McCaig and McCalla (7) that a protein-lipid complex is formed when wheat flour is made into a dough. This concept was confirmed at least in part by the observations of Olcott and Mecham (9), who found that much of the ether-extractable material of flours became "bound" (i.e., no longer extractable with ether) when flours were wetted or doughed and then dried by lyophilization. Phospholipids were bound preferentially in comparison with other flour lipids; and most of the lipids were found associated with that fraction of the gluten proteins usually referred to as glutenin.

These observations were largely restricted to flour-water doughs, however, and it was thought that other bread-dough ingredients might influence the extent of lipid binding. This has been found to be the case with salt and a polyoxyethylene stearate "softener"; their inclusion in a dough decreases binding both of total lipid and of phospholipid. Some observations also have been made on glutens washed out in the presence and absence of these ingredients; the lipid contents of the glutens followed in general the pattern expected from the observations made on doughs.

[1] Manuscript received May 26, 1952. Presented at the Annual Meeting, April, 1952.

[2] Contribution from Western Regional Research Laboratory, Albany, Calif., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Materials and Methods

The three flours used in most of the work were unbleached straight-grade flours milled from pure variety lots of wheat; all were of good baking quality. The Thatcher flour contained 0.43% ash and 16.5% protein (N × 5.7) dry basis; the Mida flour, 0.41% ash, 13.2% protein; and the Pawnee flour, 0.46% ash, 11.8% protein.

Doughs were mixed with 30 g. flour (14% moisture basis) in a small recording dough mixer (National Mfg. Co.)[3] to optimum development. Lard and a polyoxyethylene stearate preparation (Myrj-45)[3] were added as solids; salt was dissolved in a portion of the water. The doughs were frozen rapidly by pressing into thin sheets on a block of solid carbon dioxide, and lyophilized. The dried doughs were ground in a small Wiley mill equipped with a 40-mesh screen.

Ether-extractable material was determined by either of two extraction procedures: percolation of solvent through the sample held in a powder funnel over a filter paper, or extraction in a Soxhlet (18 to 20 hours) with the sample wrapped in filter paper. The two methods gave results in good agreement. An advantage of the percolation method was that a large sample (10 g.) could be used readily when a low percentage of extractable material was present; when relatively large percentages were present, however, the Soxhlet procedure was more convenient and reliable. All extracts were concentrated to about 20 ml. and filtered through medium-porosity sintered glass covered with filter aid (Celite)[3] before the final evaporation of solvent and weighing.

Total lipid contents of lyophilized glutens were determined by the Soxhlet procedure with absolute ethanol as extractant; the ethanol was then evaporated almost completely, the residue was taken up in ethyl ether, the solution filtered, the ether evaporated, and the residue weighed.

Phosphorus was determined by the method of Allen (1); total nitrogen, ash, and moisture by A.O.A.C. methods (8).

Results

Influence of Salt. The effects of varying sodium chloride additions on the binding of lipid and lipid phosphorus in Thatcher and Pawnee flour-water-salt doughs is shown in Fig. 1. In the absence of added salt, binding of lipid phosphorus was nearly complete with both flours. Binding decreased as salt was added; but the values obtained with the small amounts of extract were rather scattered and do not show clearly whether a leveling off occurred at high salt concentrations, nor whether

[3] Mention of this and other products does not imply that they are endorsed by the Department of Agriculture or that they are recommended over others not mentioned.

the flours differed in behavior in this respect. Binding of lipid was higher with the Thatcher than with the Pawnee flour, but in the case of the Thatcher flour decreases in binding continued at higher salt levels. This difference possibly is attributable to the higher protein content of the Thatcher flour.

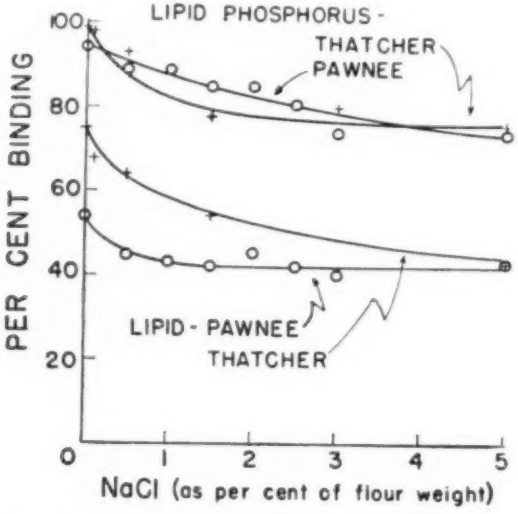

FIG. 1. Effects of sodium chloride content on the amounts of lipid and lipid phosphorus bound in doughs.

Other "Ether-Insoluble" Ingredients. No effects of sucrose, non-fat milk solids, yeast (without fermentation and with 4 hours' fermentation), potassium bromate, or malted wheat flour on lipid binding were found when these ingredients were added separately to flour-water doughs at the levels commonly used in bread production. In addition, "complete" doughs containing all the above ingredients with and without salt were compared, and the lipid and lipid phosphorus extracted differed little from the amounts obtained with the simpler flour-water and flour-water-salt doughs.

Effects of Shortening Agents (Lard and Polyoxyethylene Stearate). Two series of doughs were prepared with Pawnee flour and 2.5% sodium chloride, with lard or with a polyoxyethylene stearate product (Myrj-45) added at various levels. Both shortening agents were free of detectable amounts of phosphorus. The extents of lipid and lipid phosphorus binding observed are shown in Figs. 2 and 3.

The amounts of lipid bound (flour lipid plus lard) increased only very slightly as lard was added, while the binding of lipid phosphorus

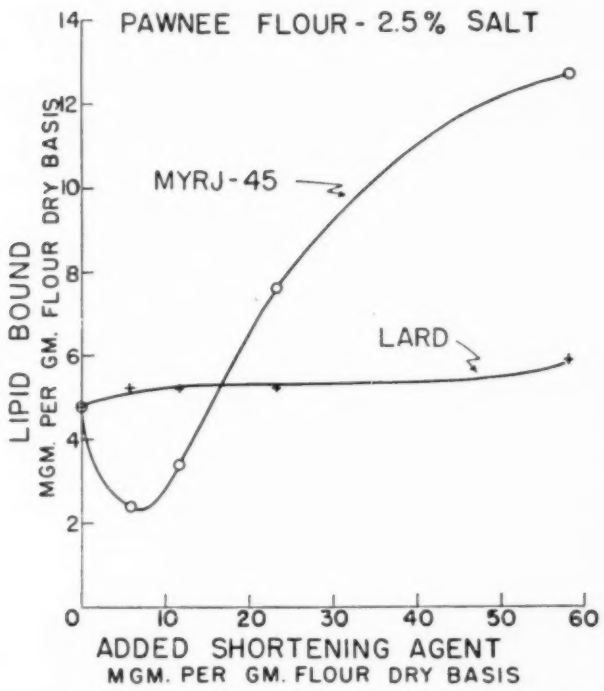

FIG. 2. Effects of lard and polyoxyethylene stearate on the amounts of lipid (flour lipid plus added shortening agent) bound in doughs.

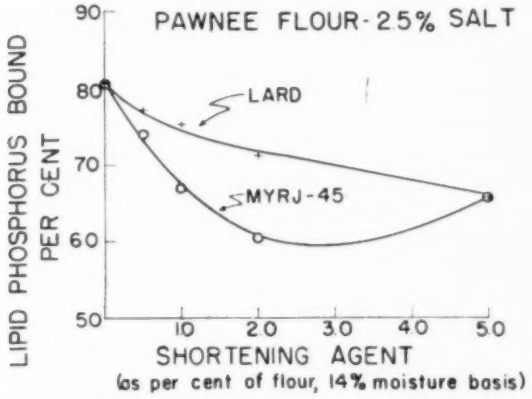

FIG. 3. Effects of lard and polyoxyethylene stearate on the amounts of lipid phosphorus bound in doughs.

decreased slightly but steadily with each increment of lard. The results indicate a limited replacement by lard of a portion of the flour phospholipid that was bound in the absence of lard.

The effects of Myrj-45 were quite different. Additions up to about 1% decreased both the total lipid and lipid phosphorus bound. With larger additions, binding increased rather rapidly; and as indicated by the 5% Myrj-45 value, the displacement of lipid phosphorus was reversed above 2% Myrj-45.

Salt and Polyoxyethylene Stearate Combinations. The results presented above show that salt decreased binding of lipid phosphorus, and that an additional decrease resulted from addition of Myrj-45. To determine whether Myrj-45 would exert similar effects at other salt levels, doughs were prepared with combinations of salt and Myrj-45 as indicated in Table I. Even in the absence of added salt, additions of Myrj-45 increased the amount of lipid phosphorus extractable.

TABLE I

LIPID PHOSPHORUS EXTRACTABLE BY ETHYL ETHER [1] FROM LYOPHILIZED DOUGHS CONTAINING SODIUM CHLORIDE AND POLYOXYETHYLENE STEARATE IN VARIOUS COMBINATIONS

Flour	Salt [2]	Myrj-45 [2]		
		0	0.5	1.0
	%	%	%	%
Thatcher	0	1	8	20
	0.5	7	41	46
	2.0	21 [3]	47	67
Mida	0	9	44	67
	2.0	36	65	81

[1] Expressed as percentage of lipid phosphorus extractable by ethyl ether from the flours.
[2] Per cent of flour weight on a 14% moisture basis.
[3] Value taken from Fig. 1.

The data in Table I in addition suggest that rather marked differences occur between flours in regard to the effects of salt and Myrj-45 in decreasing binding of lipid phosphorus. The Mida flour doughs were affected to the greatest extent; Pawnee flour doughs (Fig. 1) were least affected, at least at a salt content normal for bread doughs. The Thatcher flour, however, showed a more than additive effect of *combinations* of salt and Myrj-45. Thus 0.5% salt plus 0.5% Myrj-45 prevented binding of 41% of the lipid phosphorus, while the sum of the effects of the individual additions was only 15%.

Ether-Extracted Flour Doughs. In the work reported above, it was assumed that inclusion of salt or Myrj-45 in doughs prevented binding of a portion of lipid which was bound in flour-water doughs (and was ether-extractable in the original flour). An alternate possibility was

that some lipid not extractable from the original flour was released by additions of salt or Myrj-45.

That the latter explanation is incorrect was shown by use of ether-extracted Thatcher flour. A dough prepared with this flour and water yielded an insignificant amount of ether extract (less than 0.2 mg. per g. flour) containing no detectable amount of phosphorus. A dough containing 1% salt gave identical values. With addition of 1% Myrj-45 or 1% of both Myrj-45 and salt, the extractables weighed 67 and 60%, respectively, of the weight of added Myrj-45, but still contained no detectable amount of phosphorus. Thus salt did not release lipid not free in the original Thatcher flour, and salt, Myrj-45, or the two in combination did not release lipid phosphorus.

TABLE II

RECOVERY OF NITROGEN, LIPID, AND LIPID PHOSPHORUS IN GLUTENS

Flour	Gluten Wash Solution	Recovered in Gluten from 100 g. Flour, Moisture-Free Basis		
		N	Lipid	Lipid P
		g.	*g.*	*mg.*
Mida	Distilled water	1.44	1.36	8.4
	0.1% phosphate buffer, pH 6.8	2.07	1.26	9.2
	2% NaCl	1.96	0.87	6.3
	2% NaCl (1% Myrj-45)	1.97	0.45	1.9
Pawnee	Distilled water	1.91	1.20	6.0
	0.1% phosphate buffer, pH 6.8	1.99	1.19	6.2
	2.5% NaCl	1.98	0.89	4.4
	2.5% NaCl (0.5% Myrj-45 in dough)[1]	1.88	0.59	2.7
	Distilled water (0.5% Myrj-45 in dough)[1]	1.92	1.37	6.5

[1] Myrj-45 was added only at the time doughs were mixed; the percentages indicated are on a flour-weight basis.

Gluten-Washing Experiments. The data given in the preceding sections show that in doughs dried from the frozen state the amounts of lipid, and lipid phosphorus, made non-ether-extractable by the doughing process can be altered by additions of salt or polyoxyethylene stearate. If the gluten proteins are largely responsible for the binding of lipid, as indicated by Olcott and Mecham (9), the lipid contents of glutens washed out in the usual way presumably could be altered by the salt content of wash solutions or the addition of salts or polyoxyethylene stearate to doughs prior to the gluten washing. The results of some experiments demonstrating such effects are given in Table II.

Because the data given are for *total* lipid (*ethanol* extractable, ether soluble), a portion of flour lipid is included that was not dealt with in the studies on doughs, and this portion contains more phosphorus than

that extractable by ether from flour (unpublished observations). Even with inclusion of this additional fraction of lipid, the results give a preliminary indication that the effects of salt and Myrj-45, present during the washing of glutens, on the lipid contents of glutens correspond in a general way with their effects on lipid binding in doughs.

Discussion

Modification of flour baking behavior or of the physical properties of glutens by the addition of various types of lipids, or the removal of part of the flour lipids, has been reported several times; for examples see McCaig and McCalla (7); Sullivan, Near, and Foley (10); Kosmin (5); and Working (11). In relation to problems of baking behavior and storage stability of flour, a better understanding of the ways in which lipids exert their modifying effects would be useful. Although lipids undoubtedly can modify baking behavior by reason of their own physical properties (2), other effects may be presumed to be attributable to their combination with proteins and consequent modification of protein properties.

Little is known of the nature of the lipid-protein complexes formed upon doughing. However, it seemed some useful information might be gained from experiments of the type reported in this paper before a more basic study was attempted. The observations on the effect of salt do suggest that the binding is partly ionic in nature; this could also be inferred from the preferential binding of phospholipid.[4] The effects of polyoxyethylene stearate must be explained on some other basis. The differing behavior of lard and polyoxyethylene stearate suggests involvement of the hydrogen bonding abilities that the polyoxyethylene compound may be assumed to have.

Another implication of the results concerns the nature of glutens prepared for gluten characterization studies. These often have been separated from flour by washing out with water or the dilute phosphate buffer recommended by Dill and Alsberg (4). As indicated by the data in Table II, such glutens may be combined with more lipid, and especially phospholipid, than are glutens as they exist in bread dough; such gluten preparations accordingly may not provide a precise indication of the properties of glutens in doughs.

Evaluation of the usefulness of the lipid-binding phenomena noted in explaining various problems in baking technology requires more information than is now available, however.

[4] For discussion of the linkages which might be expected in lipid-protein complexes, and of lipoproteins in general, see (3) and (6).

Acknowledgments

We are indebted to Mr. Roy K. Durham, Mr. R. N. McCaull, The Northwest Crop Improvement Association, and Pillsbury Mills, Inc., for the Thatcher and Mida flours; to Dr. J. A. Shellenberger, Dept. of Milling Industry, Kansas State College, for the Pawnee flour; to Drs. Ali Mohammad and Walter S. Hale for preparation of the gluten samples; and to Miss Elizabeth A. McComb for phosphorus analyses.

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GRAIN STORAGE STUDIES. XII. THE FUNGUS FLORA OF STORED RICE SEED[1]

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ABSTRACT

The mold count of 12 varieties of rice from Louisiana and Surinam ranged from 500 to 66,000/g. The major portion of the count was made up of species of *Aspergillus* and *Penicillium*. Fungi were isolated from the hulls of all varieties, the principal genera being *Aspergillus*, *Penicillium*, *Fusarium*, and *Curvularia*. Fungus mycelium was present within the pericarp of all seeds examined, and various fungi were isolated from the dehulled seeds of all varieties.

Molds did not increase in rice stored in sealed bottles at moisture contents of 14% and below, nor in rice stored at higher moisture contents at temperatures of 23° and 37°F. for 21 days. At temperatures of 63° to 75°F. molds increased with increasing moisture content above 15%, and the viability of the seed decreased.

A combination of several technics probably is necessary to determine the extent to which a given lot of seed has been invaded by various molds.

The microflora on and within seeds are known to affect the quality, storage behavior, and processing of various kinds of agricultural grains. Considerable work has been done on the relation of fungi to the deterioration of stored soybeans (11, 12) wheat (3, 10), cottonseed (6), and corn (1, 2, 7, 13), but little is known about this aspect of rice storage. Rice is one of the major food crops of the world. Much of it is raised and stored in regions of high humidity, where fungus invasion of the stored seed might be expected. For these reasons it was thought that a study of the microflora of rice seed, particularly as they are related to storage of the seed, would be of some value.

The object of the work was to determine: 1. The number and kind of fungi on and within various lots of stored rice; 2. The portions of the seed in which the fungi were present; 3. The influence of moisture content, temperature, and time upon the development of fungi on and within stored seed; and 4. Their effect upon the viability of stored seed.

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Materials and Methods

Varieties and Sources of Seed. The varieties of rice, and the sources from which they were obtained, were:

From Surinam, South America, 1949 crop: varieties Skrivimankotti, Kretek, Holland, and Tjahaja.

From Louisiana, U.S.A., 1949 crop: varieties Rexoro, Fortuna, Magnolia, Zenith, and Improved Blue Rose. 1950 crop: variety Zenith.

For the storage tests, a separate lot of Zenith from Louisiana was used; this had a considerably higher total mold count than the one previously obtained.

Mold Count. The mold counts were made according to the method described by Bottomley, Christensen and Geddes (2).

Fungi within the Hulls. Hulls were removed from the seeds by rubbing the seeds between two pieces of corrugated rubber nailed to wood blocks. The hulls then were washed for two hours in running water at a temperature of about 30°C., with a small amount of detergent added every half hour, as described by Christensen (5). The hulls were then washed in two changes of sterile water, and cultured on malt-salt agar in Petri dishes. In most cases, 100 hulls were cultured from each variety.

Fungi Beneath the Pericarp. Pericarps from a number of seeds of each lot were examined microscopically. The seed was dehulled, moistened, then frozen, after which the pericarp could be removed easily with forceps. This was placed inner side up on a slide, stained with cotton blue as described by Hyde (8) and Hyde and Galleymore (9) in their work with wheat, and examined microscopically.

Fungi in the Caryopsis. The seeds were dehulled and washed in running tap water for two hours, as described above, rinsed in two changes of sterile water, and cultured on malt-salt agar.

Storage Tests. Distilled water was added to 100 g. portions of the grain to bring it to the desired moisture content, and during the first two days the seed was shaken at frequent intervals to ensure equal distribution of moisture. It was stored either in 100 g. quantities in sealed 8 oz. bottles, or in 50 g. quantities in bottles through which air at a relative humidity in equilibrium with that around the seed was drawn by means of a continuous siphon, at a rate calculated to completely replace the air in each container every 24 hours and thus prevent accumulation of carbon dioxide. The moisture content of all samples was determined by the two stage air-oven method (4) at the beginning and end of all tests. At the end of the storage tests, mold

counts were made as described above, and viability of the seed was determined by placing 100 seeds on a moist paper at room temperature and counting the number of germinated seeds after five to seven days.

Results

Total Mold Count. The total mold count of 12 samples, and the proportion of the total made up by various fungi are summarized in Table I. The count ranged from less than 500 per g. to 66,000 per g. Three samples, all from Louisiana, had a total mold count in excess of 50,000 per g. In these three, *Aspergillus glaucus* comprised more than 80% of the total. Judging from experience with other types of seed, a count of this magnitude of *A. glaucus* would indicate that some deterioration of these lots had already occurred.

TABLE I

NUMBERS AND KINDS OF FUNGI CULTURED FROM 12 VARIETIES OF RICE SEED

Variety of Rice	Source and Year of Harvest	Mold Count per Gram	Per Cent of Total Count Made Up by the Principal Genera and Species										
			Aspergillus niger	*A. glaucus*	*A. flavus*	*A. candidus*	*A. terreus*	*Penicillium* sp.	*Alternaria*	*Curvularia*	*Fusarium*	*Hormodendrum*	*Streptomyces*
Holland	Surinam 1949	900	66	5	4	5	0	13	2	0	0	2	0
Skrivimankotti	Surinam 1949	2000	24	8	4	3	37	14	5	0	2	0	0
Tjahaja	Surinam 1949	2100	25	0	3	0	1	4	52	0	12	0	0
Kretek	Surinam 1949	1900	33	15	7	0	28	6	5	0	0	0	0
Rexoro I	Louisiana 1949	400	45	5	5	5	0	40	0	0	0	0	0
Fortuna	Louisiana 1949	460	8	30	0	0	0	61	0	0	0	0	0
Magnolia I	Louisiana 1949	61,000	1	91	8	0	0	0	0	0	0	0	0
Improved Blue Rose	Louisiana 1949	300	15	23	0	0	0	62	0	0	0	0	0
Zenith	Louisiana 1949	1000	12	33	0	0	6	36	0	2	2	0	8
Early Prolific	Louisiana 1950	58,000	0	84	2	1	0	6	0	5	0	0	2
Magnolia II	Louisiana 1950	66,000	0	82	1	2		6		1	1		1
Rexoro II	Louisiana 1950	12,000											

Species of *Aspergillus* and *Penicillium* made up the bulk of the count in all of the samples. Also present were *Curvularia*, *Fusarium*, *Alternaria*, *Hormodendrum* (*Cladosporium*) and *Streptomyces*. Doubtless other fungi were present but did not show up in the dilutions used. This technic is primarily suited to disclosing the numbers and kinds of viable spores present; fungi present as only occasional portions of dormant mycelium within the hull or the caryopsis itself would not be likely to be detected.

Fungi in the Hulls. Living fungi were present in nearly 100% of the washed hulls. In the hulls cultured from four varieties from Surinam, *Fusarium* comprised 32% of the isolates, *Aspergillus niger* 31%, and *Hormodendrum* (*Cladosporium*) about 20%. As determined by total mold count, *Fusarium* was present in only two of these samples, and then in rather small numbers. Presumably, *Fusarium* was present in the hulls primarily in the form of dormant mycelium.

Of the fungi isolated from the washed hulls of the 1949 samples from Louisiana, *Aspergillus glaucus* comprised 43% of the isolates, *A. niger* 33%, *Penicillium* 8%, and *A. terreus* 8%.

Of the 1950 crop samples from Louisiana, 49% of the isolates from the washed hulls were *Curvularia*, 17% were *Fusarium*, and 14% were *Aspergillus glaucus*. The total mold count of these samples disclosed no *Fusarium*, and very little *A. glaucus*. This again illustrates the desirability of using more than one technic in determining the principal kinds of fungi present in seed. The fact that a given fungus grows from a given fragment of hull gives no information as to whether the colony arose from a single cell of mycelium, a multitude of cells, or from one or more spores. The technic is useful in determining the general prevalence of different fungi in the hull, but it does not measure accurately the amount of inoculum present.

Fungi in the Caryopsis, or Dehulled Seed. Fungi grew from more than 50% of the dehulled seed of 10 of the 12 samples tested. This indicates that internal infection of the seed itself, as distinguished from invasion of the hull or husk, is rather common. The principal molds isolated from the caryopsis, in order of prevalence, were *Aspergillus glaucus*, *A. niger*, *Curvularia*, *Penicillium*, and *Fusarium*. Most of the other fungi that were found in the seeds by the technics described above were found also in a few of the seeds in most of the samples tested. It is of interest that *A. glaucus*, the principal fungus involved in the deterioration of seeds whose moisture content is in equilibrium with a relative humidity of 65 to 75%, was the most prevalent fungus within the caryopsis of the rice seeds.

Microscopic Examination of Pericarps. Mycelium was present on the inner side of the pericarp of all seeds of all lots. Such evidence by itself indicates only that fungus invasion of the caryopsis of rice seed is prevalent; combined with evidence obtained from the different culture technics, it suggests that various molds are likely to be well established in rice seed when it goes into storage, and that they persist for some time as dormant mycelium not only within the hull but also within the pericarp.

Storage Tests. The mold counts of rice seed stored in closed bottles at four moisture contents and four temperatures for 21 days are sum-

marized in Table II. Judging from these results, the critical moisture content for the growth of molds on rice is somewhere between 14 and 16%.

The samples in closed bottles at room temperature were kept for 96 days, and the moisture content, viability, and mold count deter-

TABLE II

EFFECT OF MOISTURE CONTENT AND TEMPERATURE ON THE MOLD COUNT OF RICE SEED STORED FOR 21 DAYS[1]

Moisture Content, % (wet wt.)	Temperature °F.			
	23	37	62	70–75 (room)
	molds per g.			
12	33,000	39,000	35,000	18,000
14	45,000	37,000	37,000	11,000
16	40,000	26,000	58,000	129,000
18	23,000	25,000	70,000	304,000

[1] Original mold count approximately 40,000 per g.

mined. The results are given in Table III. The moisture content of all samples was somewhat higher at the conclusion of the test than at the beginning. At the two lower moisture contents the mold count had decreased to less than one-twentieth of the original. At 16.3% moisture, *Aspergillus glaucus* made up 100% of the count, and at 19.1% moisture *Candida pseudotropicalis* comprised 100% of the count.

TABLE III

EFFECT OF MOISTURE CONTENT ON MOLD COUNT AND VIABILITY OF RICE SEED STORED IN SEALED BOTTLES AT ROOM TEMPERATURE FOR 96 DAYS[1]

Moisture Content, % (wet wt.)	Viability, %	Mold Count per g.[1]	Percentage of Mold Count Made Up by			
			Candida pseudo-tropicalis	*Aspergillus glaucus*	*Penicillium*	*Hormo-dendrum*
13.6	95	1400		43	14	43
14.3	88	1800		11	78	11
16.3	67	720,000		100		
19.1	35	6,320,000	100			

[1] Original mold count approximately 40,000 per g.

The mold counts at both of the higher moisture contents were very much higher than the original ones, and the viability of the seed was lower.

Aliquots of 50 g. each of rice seed were stored for 18 days in aerated bottles at constant relative humidities to maintain moisture contents in the seed of 15.3 to 18.9%. Mold counts and viability of the seed

TABLE IV

EFFECT OF MOISTURE CONTENT ON VIABILITY AND MOLD COUNT OF RICE STORED FOR 18 DAYS IN AERATED CONTAINERS

Moisture Content, % (wet wt.)	Viability, %	Mold Count per g.	Per Cent of Total Mold Count Made Up by				
			Aspergillus glaucus	*A. flavus*	*Penicillium*	*Hormodendrum*	*Curvularia*
10 (before storage)	98	2500	24	5	16	5	50
15.3	93	12,000	100				
16.7	89	360,000	100				
18.9	81	5,000,000	100				

are given in Table IV. In this test the mold count increased and the viability of the seed decreased slightly at a moisture content of 15.3%. At the higher moisture contents the increase in mold count and decrease in viability were both more marked. In all of the aerated samples *Aspergillus glaucus* comprised 100% of the count.

Discussion

The kinds of fungi on and within samples of rice seed from the southern United States and from Surinam, South America were, in general, very similar to those found in other kinds of agricultural seeds, with the exception that *Curvularia* was considerably more prevalent.

Some of the samples of rice seed had a high count of *Aspergillus glaucus*, a group species that is known to infect seeds and other materials whose moisture content is in equilibrium with a relative humidity of 65 to 75%. Its presence in fairly large numbers in some of these samples would suggest that they had been moist enough in storage for this fungus to increase. The data in Table IV indicate that as *A. glaucus* increases, the viability of the seed decreases. This suggests that this fungus gradually weakens and kills the germ.

The different methods used to determine moldiness of the seed, or the degree or extent to which the seed had been invaded by various molds gave different results. No one of the technics used in this study is presumed to give an adequate answer of the degree to which seed has been invaded by certain fungi. Each of them has some utility in determining the extent to which various portions of the seed have been invaded by various molds. If a given seed lot has a very high count of a species of fungus known to invade seed and to cause loss of viability in storage, this is presumptive evidence that the seed is deteriorating or has already deteriorated. If the technics further disclose that a given mold is present in the hull or husk, but not in the caryopsis, it can be presumed that no really serious deterioration has occurred. If a fungus known to grow at the moisture contents normally encountered

in storage is found in the caryopsis of a large proportion of the seeds, and if the total count is high, and the viability of the seed is below normal, it may be presumed that this lot of seed is a poor storage risk. At present there is no microbiological evaluation of any agricultural seed when it goes into storage. If practical methods of microbiological evaluation of the quality and storability of seeds could be developed, they might be of value for many kinds of seed.

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ABSENCE OF MESO INOSITOL IN PURIFIED AMYLASE PREPARATIONS FROM DIFFERENT SOURCES [1]

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ABSTRACT

A number of amylase preparations from different sources have been assayed for inositol by a microbiological method. No trace of inositol was found in crystalline pancreatic amylase; in highly purified preparations of beta amylase from barley; in those of taka amylase, the alpha amylase formed by the mold *Aspergillus oryzae;* or in those of gluc amylase, a glucose forming amylase produced by the mold *Rhizopus delemar.* Inositol was present in pancreatin and in unpurified extracts and concentrates used as sources of the amylases. This inositol was separated from the amylases during their purification.

No evidence has been obtained that inositol has any influence upon the stability or upon the activity of any of the amylases investigated.

In 1944, Williams, Schlenk, and Eppright (17) reported the presence of *i*-inositol in preparations of purified pancreatic amylase. In 1948, Lane and Williams (10) reported indirect confirmatory evidence for this finding. In 1949, Fischer and Bernfeld (8) reported the absence of inositol in their crystalline pancreatic and salivary amylases.

In view of these reports and of the importance of the general subject of the function of inositol in nature, it seemed worthwhile to assay a number of amylase preparations from different sources for inositol and also to ascertain whether the activities or the stabilities of highly purified amylases might be influenced by the presence of inositol.

Highly purified crystalline pancreatic amylase (4), prepared by a method quite different from that reported by Meyer *et al.* (11, 12), was assayed for inositol. Also assayed for inositol were highly purified preparations of an amylase from *Aspergillus oryzae*, taka amylase (6, 9), of beta amylase from barley (5) and of a glucose forming amylase from *Rhizopus delemar*, gluc amylase (13). For comparison, assays for inositol were also carried out with pancreatin from which the crystalline pancreatic amylase had been prepared and with crude concentrates and partially purified preparations of the other amylases studied.

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Materials and Methods

Inositol was determined by a slight modification of the microbiological assay procedure described by Atkin, Schultz *et al.* (1, 2, 3) using yeast culture No. 4228, a strain of *Saccharomyces carlsbergensis* whose growth responds to the presence of inositol in the medium.

The procedure recommended by Atkin, Schultz *et al.* (1, 2, 3) was changed in these respects: an Evelyn photoelectric colorimeter (7) instead of a Lumetron 400 was used for measuring the turbidities of the yeast cultures; measurements of the turbidities were made 15 hours after incubation and the readings were recorded as per cent transmission of light. The filter was number 440. Sucrose was used in the medium instead of dextrose. Under the conditions of these experiments, results with sucrose were more consistently reproducible than those with dextrose.

The calculation of results was based upon standard control curves, prepared daily for the growth of the yeast incubated with increasing concentrations of inositol. Before being assayed for inositol, the amylase preparations were subjected either to autolysis, under a layer of benzene, with subsequent treatment with acetic acid as recommended by Williams *et al.* (18, 19) or to hydrolysis with hydrochloric acid as recommended by Atkin, Schultz *et al.* (1).

Results and Discussion

Reference Curves with Inositol. Typical reference control curves obtained with yeast cultures incubated with increasing concentrations of inositol and obtained at different times are given in Fig. 1. These curves emphasize the need for the daily control curve (3, 18, 19).

Control measurements with solutions of inositol, containing 100 mg. inositol per 100 ml., showed that the autolysis or the hydrolysis treatments of the amylase preparations would have no effect upon any inositol they might contain.

Pancreatic Amylase. Hog pancreatin, finely ground defatted pancreas glands from the hog, was assayed for inositol. It was found to contain an average of 5 μg. of inositol per mg. if assayed after autolysis (18, 19), and an average of 7 μg. per mg. if assayed after hydrolysis with hydrochloric acid (1). No evidence of inhibition of the growth of the yeast was observed with autolyzed or with hydrolyzed pancreatin. Inositol added to the medium with autolyzed or with hydrolyzed pancreatin was accounted for quantitatively in the subsequent assay.

Highly active crystalline pancreatic amylase (4, 14, 15, 16) obtained from the pancreatin referred to above, gave no evidence of the presence

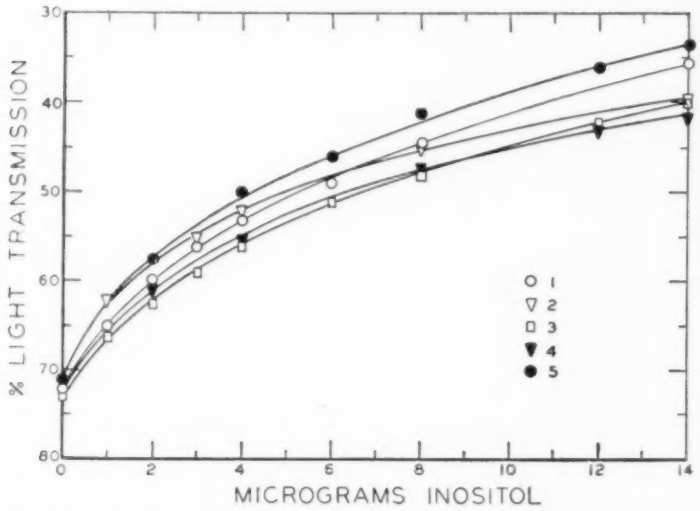

FIG. 1. Growth, 15 hours after incubation, of yeast No. 4228, on inositol free medium and on that medium treated with increasing concentrations of inositol. Curves are for cultures grown on different days.

of inositol either after autolysis (Table I) or after hydrolysis with hydrochloric acid (Table II). Inositol could be accounted for quantitatively if added to the medium with the autolyzed crystalline pancreatic amylase (Table I).

TABLE I

ASSAY FOR INOSITOL OF CRYSTALLINE PANCREATIC AMYLASE[1] AFTER AUTOLYSIS. GROWTH OF YEAST NO. 4228

Tube	Inositol Added per Tube	Autolyzed Amylase	Inositol	
			Total	Present in Amylase
no.	*μg.*	*mg.*	*μg.*	*μg./mg.*
1	0	0	0	0
2	0	0.54	0	0
3	0	1.08	0	0
4	0	1.62	0	0
5	4	0	4.0	0
6	4	0.54	3.4	0
7	4	1.08	3.8	0
8	4	1.62	3.8	0

[1] The crystalline pancreatic amylase (4) produced 16,000 times its weight of maltose equivalents in 30 minutes at 40° from 1% starch (14) at a concentration of 1.3×10^{-5} mg. amylase per mg. starch.

Crystalline pancreatic amylase that had been hydrolyzed with hydrochloric acid caused a slight inhibition of the growth of the yeast (Table II). This inhibition presumably was caused by an amino acid or amino acids set free during the hydrolysis of the amylase (4).

TABLE II

ASSAY FOR INOSITOL OF CRYSTALLINE PANCREATIC AMYLASE AFTER HYDROLYSIS WITH HYDROCHLORIC ACID. GROWTH OF YEAST NO. 4228

Tube	Inositol per Tube	Hydrolyzed Amylase	Inositol	
			Total	Present in Amylase
no.	*μg.*	*mg.*	*μg.*	*μg./mg.*
1	0	0	0	0
2	0	0.46	0	0
3	0	0.92	0	0
4	0	1.38	0	0
5	0	1.84	0	0
6	4	0	4.0	0
7	4	0.46	3.0	0
8	4	0.92	2.3	0
9	4	1.38	1.5	0
10	4	1.84	1.3	0

Although this inhibition interfered with the quantitative determination of inositol added to the medium in the presence of the hydrolyzed amylase, it did not obscure entirely the evidence that inositol was present. Therefore, it was concluded that crystalline pancreatic amylase contains no inositol, free or combined, that is demonstrable by this microbiological method. Fischer and Bernfeld (8) also did not find inositol in their crystalline pancreatic amylase.

Preparations of Highly Purified Alpha Amylase from Aspergillus oryzae, Taka Amylase. Commercial concentrates of amylase from extracts of the mold, *Aspergillus oryzae*, were assayed for inositol. They averaged approximately 10 μg. inositol per mg. of concentrate. On

TABLE III

ASSAY FOR INOSITOL OF HIGHLY PURIFIED AMYLASE FROM *Aspergillus oryzae*[1]. GROWTH OF YEAST NO. 4228

Tube	Inositol Added per Tube	Autolyzed Amylase Preparation Added per Tube	Inositol	
			Total	Present in Amylase Preparation
no.	*μg.*	*mg.*	*μg.*	*μg./mg.*
1	0	0	0	0
2	0	0.59	0	0
3	0	1.18	0	0
4	0	1.77	0	0
1	4	0	4	0
2	4	0.59	3.5	0
3	4	1.18	3.5	0
4	4	1.77	4.2	0

[1] The purified taka amylase (9) produced 2,200 times its weight of maltose equivalents in 30 minutes at 40° from 1% starch (6) at a concentration of 9.04×10^{-5} mg. per mg. starch.

the other hand, preparations of highly purified alpha amylase, taka amylase, obtained from these concentrates (9) gave no evidence of the presence of inositol even when concentrations as high as 7.7 mg. of the autolyzed or hydrolyzed preparations were used for the assay. In this case, there was no inhibition of growth of the yeast by the autolyzed or by the hydrolyzed amylase preparations, and inositol added to the medium was accounted for quantitatively in the assay. Typical data are given in Table III.

Preparations of Highly Purified Gluc Amylase, Obtained from Extracts of Rhizopus delemar. Commercial concentrates of amylase from extracts of the mold, *Rhizopus delemar*, were assayed for inositol. They averaged approximately 11 μg. inositol per mg. of concentrate. On the other hand, preparations of highly purified gluc amylase, a glucose forming amylase (13) obtained from these concentrates, gave no evidence of the presence of inositol even when 9.5 mg. of the preparation were used for the assay. Typical data are given in Table IV.

TABLE IV

ASSAY FOR INOSITOL OF HIGHLY PURIFIED GLUC AMYLASE[1] FROM *Rhizopus delemar*. GROWTH OF YEAST NO. 4228

Tube	Autolyzed Amylase Preparation Added	Inositol Found
no.	*mg.*	*μg.*
1	0	0
2	3.17	0
3	6.24	0
4	9.51	0

[1] The highly purified gluc amylase (13) produced 500 times its weight of glucose from 1% starch in 30 minutes at 40° (13) at a concentration of 5.7×10^{-4} mg. per mg. starch.

Highly Purified Beta Amylase. A preparation of highly purified beta amylase (5) also was assayed for inositol. This preparation had an exceedingly high amylase activity and produced 12,000 times its weight of maltose from 1% starch in 30 minutes at 40°C. at a dilution of 1.8×10^{-5} mg. amylase preparation per mg. starch. Concentrations of 1 mg. of this beta amylase preparation in the yeast cultures had no appreciable influence on the growth of the yeast; they gave no evidence of the presence of inositol. Higher concentrations of the amylase preparations could not be assayed for inositol by the method employed because they caused the formation in the yeast cultures of precipitates that interfered with the turbidity measurements of the growth of the yeast.

Stability and Activity. Solutions of crystalline pancreatic amylase, (4), and solutions of highly purified preparations of taka amylase (9) or

of beta amylase (5) were held at unfavorable temperatures and at unfavorable hydrogen ion activities in the presence and in the absence of inositol. In each case, the same losses of amylase activity occurred in the presence and in the absence of inositol. Therefore, it is concluded that inositol has no appreciable influence upon the stability of these amylases when they are exposed to these unfavorable conditions. Similarly, hydrolyses of starch in the presence and in the absence of inositol led to the conclusion that inositol does not influence the action of these amylases. The inositol that accompanies these amylases in nature does not appear to be essential to their action or stability. In this connection, it is of interest to note that Schwimmer and Balls (20) found that inositol was absent from and had no influence on crystalline malt alpha amylase.

Acknowledgments

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THE DETERMINATION OF PANTOTHENIC ACID IN WHEAT [1]

FAWZI Y. REFAI [2] and BYRON S. MILLER

ABSTRACT

The assay of calcium pantothenate in 16 varieties of wheat representing eight species grown under similar environmental conditions, was performed using a modified chemical method as well as the generally accepted microbiological assay method. The modification of the chemical method consisted of the extraction of the pantothenates from wheat with methanol instead of water. Possible interfering compounds such as sterols and certain fat-soluble vitamins, which are soluble in methanol and which yield insoluble hydrazones after oxidation with potassium permanganate, had no effect on the analysis of calcium pantothenate under the conditions employed. Recovery of added calcium pantothenate using the modified chemical method averaged 98.9%. A comparison of the mean difference in the assay values obtained by the two methods showed that the chemical method yielded consistently higher results (averaging 3%). The range of calcium pantothenate in the samples assayed was 8.9 to 20.6 μg./g. of wheat. Significant differences were found in the calcium pantothenate content of different species and of different varieties within species.

During the last few years pantothenic acid has drawn the increasing attention of scientists due to its role in intermediary metabolism. Pantothenic acid is known to occur in nature in several forms. These include free pantothenic acid, coenzyme A, pantothenic acid conjugate,

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[2] Chemical Section, Ministry of Agriculture, Cairo, Egypt. Presently a member of an Egyptian Official Mission for graduate study at Kansas State College.

protein-bound pantothenic acid and various forms which do not have coenzyme A activity.

Biological and microbiological methods are widely accepted for pantothenic acid assay. As shown by Lipmann *et al.* (5) coenzyme A is not broken down to release pantothenic acid by any means except autolysis, treatment with intestinal phosphatase and pigeon or chick liver extract. Both enzymes are essential since it has been shown that 20 to 30% of the pantothenates is released by dephosphorylation and about 70% by the action of freshly prepared extract of chick liver acetone powder (8). Using this technic, free pantothenic acid is released from the bound forms and the microbiological assay values are in agreement with those obtained by biological assay.

Recently Szalkowski, Mader and Frediani (13) developed a chemical method of analysis based on the hydrolysis of calcium pantothenate and its cleavage to beta alanine and alpha, gamma-dihydroxy-beta, beta-dimethylbutyric acid. Beta alanine, when oxidized with potassium permanganate in the presence of potassium bromide under properly regulated conditions, yields an insoluble hydrazone upon treatment with 2,4-dinitrophenylhydrazine. The calcium pantothenate is estimated by dissolving the dinitrophenylhydrazone in pyridine, diluting with sodium hydroxide and measuring the resulting blue color spectrophotometrically at 570 mμ. Certain interfering compounds, with the exception of ascorbic acid, are eliminated by chromatographic adsorption on an aluminum oxide column (13).

The vitamin content of wheat is known to vary with the variety and environmental conditions. Several workers (1, 2, 4, 6, 7, 11, 14) have determined the calcium pantothenate content of wheat and wheat products employing either biological or microbiological methods of assay. It has been the object of this work to determine by chemical as well as microbiological methods the calcium pantothenate content of several species and varieties of wheat grown under comparable environmental conditions.

Materials and Methods

Sixteen samples of wheat comprising eight species from the 1950 crop grown at Brawley, California, under comparable environmental conditions were assayed for calcium pantothenate. The samples were prepared for assay by grinding in a Wiley mill to pass a 1 mm. wire screen.

The chemical method used in this work was the same as that outlined by Szalkowski *et al.* (13) except that methanol was used to extract the pantothenates. Three portions of wheat, 8, 12, and 16 g., were extracted separately. Each portion was suspended in 100 ml.

of absolute methanol, shaken vigorously and centrifuged for 10 minutes or until a clear solution was obtained. A 25 ml. aliquot of the clear solution was chromatographed, eluted with 18 *N* sulphuric acid, oxidized and measured as described by Szalkowski *et al.* (13). A typical standard curve obtained for pure calcium pantothenate using the modified chemical method, is shown in Fig. 1. Calcium pantothenate used

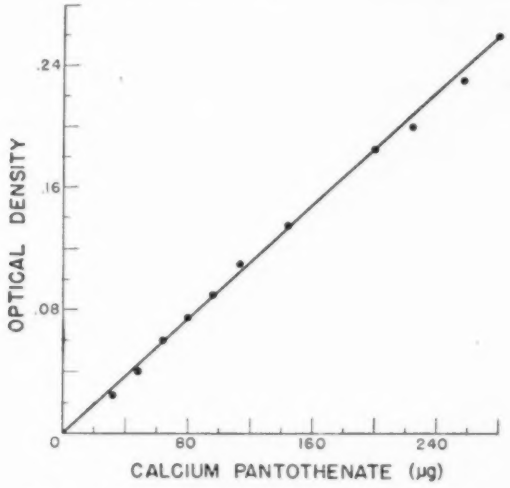

FIG. 1. Calibration curve using the modified chemical method.

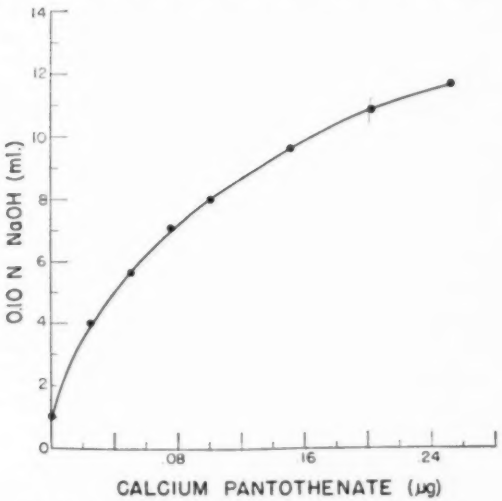

FIG. 2. Calibration curve using the microbiological method.

for preparing the calibration curve for both the chemical and microbiological methods was obtained from Merck and Co., Inc.

The microbiological method outlined by Skeggs and Wright (9) was used without modification. The test organism, *L. arabinosus* 17–5, was obtained from the American Type Culture Collection, Georgetown University, School of Medicine, Washington, D. C. Pantothenates were released by digesting wheat samples with alkaline phosphatase and chicken liver extract (8). The enzyme preparation contributed as much as 1.10 μg. of calcium pantothenate in 0.05 ml. of liver extract, or 0.011 μg. per each assay. This was equivalent to about 25% of the calcium pantothenate content of the wheat sample. A typical calibration curve is shown in Fig. 2.

Results and Discussions

Calcium pantothenate was determined in a trial sample of wheat using the chemical method outlined by Szalkowski *et al.* (13). The amount of calcium pantothenate thus measured was about 500 μg./g. of wheat, although values reported in the literature ranged between 5 and 19 μg./g. (1, 2, 4, 6, 7, 11, 14). This high result indicated the possible interference of compounds such as carbohydrates, thiamine hydrochloride, niacin and niacinamide (13). The presence of carbohydrate material in the chromatographic column was demonstrated by a positive Molisch test and by the development of a dark brown color on boiling the eluate with the sulphuric acid used to hydrolyze the calcium pantothenate.

The calcium salts of pantothenic acid have been described by Stiller *et al.* (10) as microcrystalline, hygroscopic powders which are readily soluble in water and the lower alcohols. Ford (3) observed that calcium pantothenate was soluble to the extent of more than 50 g./100 ml. of methanol. Methanol instead of water was used, therefore, to extract the pantothenates from wheat. No data are available concerning the solubility of pantothenic acid derivatives in methanol.

Certain sterols and fat-soluble vitamins are soluble in methanol and yield insoluble dinitrophenylhydrazones after oxidation with potassium permanganate. However, cereals are poor sources for the fat-soluble vitamins A and K; furthermore, these vitamins together with carotenoids, although soluble in methanol, are not adsorbed on the alumina column due to the higher polarity of methanol (12). To test the effect of soluble lipid material on the results, a 10 g. sample of wheat was extracted with petroleum ether, and the extract dissolved in 15 ml. of methanol. Aliquots of this solution were added to both wheat and the calcium pantothenate standard solution. The results

obtained showed that petroleum ether-soluble lipid material did not affect the determination of calcium pantothenate in wheat.

The recovery of calcium pantothenate, using different levels of both wheat and calcium pantothenate and the chemical method of analysis, was 98.9 ± 2.9% for three different analyses. The standard error of a single determination based on nine determinations of pure calcium pantothenate solution containing 80 μg./ml. was 3.13 μg./ml. The standard error based on seven determinations of calcium pantothenate contained in 8 g. of wheat (144.32 μg.) was 6.5 μg.

The calcium pantothenate content of the different species of wheat as determined by both the chemical and microbiological methods is recorded in Table I. Chemical values are the average of three separate analyses of three different levels in duplicate. The microbiological values are the average of two analyses each made with four different levels in duplicate.

TABLE I

THE CALCIUM PANTOTHENATE CONTENT OF WHOLE WHEAT

Species and Varieties of Wheat	Crude Protein[1]	Calcium Panthothenate[1]		
		Chemical Method	Microbiological Method	Difference
	%	μg./g.	μg./g.	
T. monococcum	12.9	12.2	11.4	0.8
T. diococcum, vernal	11.7	20.5	20.6	−0.1
T. orientale	8.8	10.1	9.8	0.3
T. pyramidale	8.1	12.0	10.7	1.3
T. persicum	9.2	11.2	10.1	1.1
T. durum				
Stewart	8.7	12.2	11.5	0.7
Pentad	10.3	14.3	13.7	0.6
T. sphaerococcum	11.8	10.1	10.1	0.0
T. vulgare				
Baart (white)	10.5	9.8	8.9	0.9
Federation (white)	9.9	11.5	11.2	0.3
Dawson (white)	13.5	16.7	16.1	0.6
Thatcher (Hard Red Spring)	11.3	11.2	11.0	0.2
Marquis (Hard Red Spring)	12.6	17.1	16.4	0.7
Trumbull (Soft Red Winter)	14.8	19.9	19.3	0.6
Comanche (Hard Red Winter)	12.6	15.3	14.6	0.7
Red Chief (Hard Red Winter)	11.8	14.8	14.5	0.3

[1] Expressed on a 14.0% moisture basis.

All results were subjected to a statistical analysis. None of the tested sources of variation other than species and varieties within species showed any significant effects for the chemical method of determination. For the microbiological method—species, variety in species and the interaction of variety in species × levels were significant. This interaction indicated that the relative differences among

the varieties were not consistent from one level to another and suggests a disadvantage of the microbiological method.

A comparison of the mean differences between the two methods (Table I) shows that the chemical method yields consistently higher values. The difference ranged from 0.5 to 10% of the mean, with the average difference being about 3%.

The comparison of the estimates of variance for the corresponding sources of variation for the two methods showed homogeneity, which permitted combination of the entire set of results into a single analysis of variance. In the pooled analysis, the mean square between methods was considerably larger than any of the estimates of variance involving the interactions of sampling with other variables. This indicates that the likelihood of obtaining such differences due to chance variation is exceedingly small. Differences due to species and to varieties within species were also highly significant.

TABLE II

CALCIUM PANTOTHENATE CONTENT IN WHEAT AS DETERMINED BY DIFFERENT WORKERS

Source of Determination		Calcium Pantothenate	No. of Samples	Methods of Assay
		μg./g.		
This work		9.8–20.5	16	Chemical
		8.9–20.6	16	Microbiological
Atkin *et al.*	(1)	8.3– 8.9	3	Microbiological
Cheldelin & Williams	(2)	13.1	1	Microbiological
Jukes	(4)	12.2	2	Chick assay
Loy	(6)	9.8	1	Microbiological
Moran & Drummond	(7)	5.5	1	Microbiological
Strong *et al.*	(11)	9.0	1	Microbiological
Teply *et al.*	(14)	9.9–19.0	55	Microbiological

It has been known that the enzymatic treatment employed prior to microbiological assay releases all the bound forms of pantothenic acid (8), thus making free pantothenic acid available for the microorganism. The similar results obtained by the chemical method appear to indicate the solubility in methanol of the free as well as the bound forms of pantothenic acid. The somewhat higher results obtained for the chemical method may indicate the presence of other compounds which are soluble in methanol and which give the same color test. Further investigation is needed to test the solubility in methanol of bound forms of pantothenic acid and the insolubility of other beta alanine derivatives.

A comparison of the results obtained in this investigation and those reported in the literature is shown in Table II. Teply *et al.* (14) found that neither varietal nor environmental differences had any significant

effect on the amount of calcium pantothenate present in four varieties of dark hard winter wheat grown at four locations. Values for miscellaneous varieties representing all classes of *T. vulgare* grown in Kansas, Minnesota, Oklahoma, Texas and Washington indicated that genetic differences were of little consequence in affecting the calcium pantothenate content of soft red winter wheat, while environmental differences had considerable effect.

The quantities of calcium pantothenate obtained in the present work by both the chemical and microbiological methods of analysis agree well with the values reported by Teply *et al.* (14). Values reported by other workers (1, 2, 4, 6, 7, 11) are also in good agreement. The largest sources of variation in the results were those due to the differences between species and between varieties within species. Since these samples were grown under the same environmental conditions, the large differences between the calcium pantothenate content of the species and varieties within species may be due to genetic variation. Further work should be done employing selected species and varieties grown at several locations for a number of years.

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A NOTE ON THE INFLUENCE OF CHLORINE DIOXIDE ON THE SOLUBILITY OF THE WHEAT FLOUR PROTEINS[1]

J. D. JACOBS[2]

Several years ago Jorgensen (1) reported that the addition of relatively large quantities of potassium bromate to wheat flour depresses the solubility of the flour proteins in water, and he postulated that the action of this reagent as a flour improver is related to this phenomenon. Chlorine dioxide has come into extensive use as a flour improver and experiments were undertaken to determine whether it also decreases flour protein solubility.

The experiments were conducted with two untreated flours (76% extraction) of the following composition:

Sample No.	*Nature of Wheat Mix*	*Flour Composition (14% moisture basis)* *Protein*	*Ash*
1	65% hard winter, 10% Northern spring, 25% Dutch	9.8	0.63
2	65% hard winter, 5% Northern spring, 12% Hungarian, 9% Australian, 9% Dutch	10.2	0.62

Fifty-gram samples of each flour were treated in duplicate with varying levels of chlorine dioxide employing a slight modification of the method described by Parker and Fortmann (2). The chlorine dioxide, generated by interacting solutions of ammonium persulfate and sodium chlorite, was aspirated over the flour contained in a horizontal glass tube (40 × 2.5 cm.). The amount of chlorine dioxide used was determined by titration of the generating solution with acidified potassium iodide solution before and after aspiration. The water-soluble proteins were extracted by immersing glass tubes (26 × 2.8 cm.) containing 10 g. of flour dispersed in 50 ml. of water in a thermostat at 30°C. The tubes were shaken at 20 minute intervals and after one hour, 50 ml. additional water was added, the tubes shaken, cooled to about 15°C. and, after settling, the solids were removed by centrifugation and filtration. The nitrogen content of 25 ml. of the clear filtrate was determined by the Kjeldahl procedure. The pH of several of the extracts was measured and the diastatic activity of a number of the flour samples was determined by the method described by van der Lee (3).

[1] Manuscript received December 28, 1951.
[2] N. V. Meelfabrieken der Nederlandsche Bakkerij, Rotterdam, Holland.

TABLE I

EFFECT OF TREATMENTS WITH CHLORINE DIOXIDE ON THE WATER-SOLUBILITY OF THE FLOUR PROTEINS

Chlorine Dioxide Treatment	Water-Soluble Proteins Per Cent of Total		Maltose Figure		pH	
	Flour 1	Flour 2	Flour 1	Flour 2	Flour 1	Flour 2
g./100 kg.			%	%		
0.0	14.8	14.7	2.6	1.9	6.12	5.85
1.5	15.0	—	—	—	—	—
3.0	14.7	—	2.6	1.9	—	—
6.0	14.9	14.2	—	—	—	—
9.0	14.7	14.2	—	—	—	—
18.0	15.2	14.7	—	—	—	—
30.0	15.8	15.4	—	—	5.80	5.76
90.0	16.5	17.0	2.5	1.7	5.80	5.70

The results of these tests (Table I) show that high dosages of chlorine dioxide increase the water-solubility of the flour proteins. This increase is evident at a treatment of 30 g./100 kg., which is approximately a fifteen-fold excess of the optimal treatment of these flours for baking purposes. With these high treatments, the flour assumed a faint pink color. These data are in marked contrast to the effect of potassium bromate found by Jorgensen. Potassium bromate depressed the protein solubility when dosages of from 60 to 200 times the optimal levels for flour improvement were used.

The diastatic activity of the flours was not influenced by chlorine dioxide and the pH decreased only very slightly, even after adding excessive amounts.

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3. VAN DER LEE, G. Zur Methodik der Bestimmung der diastatischen Kraft des Mehles. Novadel Mitteilungen, Nov. 1929, p. 108. Heft 11. Ed. by Intern. Oxygenium Mij. "Novadel," Deventer, Holland.

COMMUNICATION TO THE EDITOR

A Paper Chromatography Method for Quantitative Determination of Cystine in Wheat Gliadin Hydrolysates[1]

Dear Sir:

Recently a method [2] has been developed for the quantitative estimation of amino acids after paper chromatography separation, based on the localization of the spot corresponding to each amino acid by ultraviolet fluorescence, cutting out the spots, and development of the ninhydrin color reaction when the piece of paper with the amino acid is immersed in suitable amount of liquid in a small volumetric flask.

This method may easily be applied to the determination of cystine-cysteine in proteins, for instance in hydrolysates of wheat gliadin. Its chief advantages are its simplicity and the possibility of performing at the same time a series of routine determinations on other amino acids.

A one-dimensional paper chromatography suffices for the estimation of cystine, since this amino acid easily separates from the others using as solvent water saturated butanol-acetic acid (4:1). With this solvent and under the conditions used the R_F values for cystine and cysteine are respectively 0.12 and 0.13, while the other amino acids have greater values, the next one being that for lysine, whose R_F is 0.17.

Cystine determinations of wheat gliadin may be performed as follows.

One gram of the protein is hydrolyzed by refluxing with 20 ml. of 5 *N* hydrochloric acid for about 30 hours, the exact time being ascertained either by Van Slyke amino nitrogen determination or by cystine estimation until a maximum value is reached. The excess hydrochloric acid is removed by concentration of the hydrolysate under reduced pressure at a temperature of not more than 50°C., until a thick sirup results, the process being repeated twice. The sirup is then quantitatively transferred to a 25 ml. volumetric flask and kept in a refrigerator.

For the chromatography we employ Whatman No. 2 filter paper sheets in a box of 75 × 75 × 20 cm. A series of sheets is placed side by side, suspended from glass clips and the solvent is allowed to

[1] Work done at the Instituto de Zoologia, Universidade de Coimbra, and aided in part by a grant from the Instituto para Alta Cultura, Lisboa.

[2] Pereira, A., and Serra, J. A. Quantitative microdetermination of amino acids after paper chromatography. Science **113**: 387–388 (1951).

ascend by capillarity. The solution with the amino acids is placed as a small measured droplet at a distance of 5 cm. from the bottom of the sheet; 0.1 ml. and 0.001 ml. divided, precision grade pipettes are employed for this purpose, a quantity of solution of 5, 10 or 15 μl. being used for each single determination.

A series of measured droplets are placed at 4 or 5 cm. intervals so that a sheet of 40 cm. allows simultaneously 7 or 8 determinations. Drops of the hydrolysate are alternated with drops of a standard solution. This latter is made by dissolving known amounts of cystine and the other amino acids in about the same concentrations as in the hydrolysate of the protein. Of course, each determination is made at least in duplicate but it is better to perform three or four determinations simultaneously and to obtain the mean.

The drops are allowed to dry at room temperature and the sheet is then hung in the solvent saturated chromatography box, with the bottom immersed 0.5 cm. in the solvent. The solvent is prepared by saturating a mixture of 4 volumes of butanol and 1 volume of glacial acetic acid with water.

A time of 48–60 hours development and a 32–36 cm. solvent boundary dislocation were found satisfactory for cystine-cysteine separation from the mixture. Afterwards the sheets are air dried for 24 hours at room temperature with the aid of a fan to eliminate all the solvent. The sheets are then heated in an oven at 80–100°C. for not more than 5 minutes.

The method of Pereira and Serra[2] is then applied. As the cystine-cysteine possesses an R_F very different from that of the other amino acids, no difficulty is generally encountered in marking the corresponding spot. However, in case it is found necessary, the cystine-cysteine spot may easily be recognized by running side by side in the chromatogram, the hydrolysate and the hydrolysate with added cystine; the cystine spot appears in the latter case enlarged in relation to the spot of the hydrolysate only when a ninhydrin solution is sprayed upon the sheet. For the quantitative determination, the spot corresponding to cystine-cysteine is lightly marked under ultraviolet light with a pencil and cut out with the aid of scissors and a forceps (avoid touching it with the fingers). The spots are placed in 10 ml. dried volumetric flasks. The paper may be subdivided if necessary.

For the reaction, 0.25 ml. of a recently made 1% ninhydrin solution in water and an equal volume of a pH 7 veronal-hydrochloric acid buffer is added. The buffer is prepared by mixing 5.36 vols. of 0.1 *M* sodium veronal with 4.64 vols. of 0.1 *N* hydrochloric acid.

To develop the color, the glass-stoppered flasks are immersed (to the neck) in a bath at a temperature of 103–105°C. for 15 minutes,

with occasional shaking during this time. The bath is prepared by saturating water with common sodium chloride. After this time, the flasks are promptly immersed in tap water for 5 minutes and filled to the mark with distilled water, shaken well, and after another 5 minutes the color is measured in a suitable colorimeter using a 550 mμ. filter. A Lumetron colorimeter and a microcell 2 cm. thick were employed for this purpose. The temperature of 103–105°C. markedly improves the reaction, increasing its sensitivity.

TABLE I

RECOVERY OF CYSTINE ADDED TO THE GLIADIN HYDROLYSATE

Amount of Cystine in the Hydrolysate	Cystine Added	Total Cystine Found	Recovery
μg.	μg.	μg.	%
5	2.5	7.8	104
5	5	9.3	93
10	2.5	12.7	101
10	5	14.7	98
			Mean 99

For routine determinations, standard curves with known amounts of cystine in a mixture of the other amino acids in proportions similar to those found in the protein hydrolysate are prepared. The quantities to be determined must fall in the part of the curve in which the color is proportional to the amount of cystine present.

In order to test the accuracy of the method recovery assays were performed and the results presented in Table I.

TABLE II

Wheat Gliadin	Cystine Found	N	In the Literature	
			Cystine	N
	%	%	%	%
Determination 1	2.37		2.74[1]	—
Determination 1	2.67		2.6[2]	17.1
			2.4[2]	17.1
Determination 2	3.26			
Determination 2	3.02			
Mean	2.83	17.5		

[1] Baernstein, H. D. The sulfur distribution in proteins. J. Biol. Chem. **97**: 669–674 (1932).
[2] Bailey, K. The sulfur distribution of proteins. Biochem. J. **31**: 1396–1405 (1937).

This method has been applied to a sample of gliadin, prepared according to the method of Dill and Alsberg[3] from *Quaderna* wheat

[3] Dill, D. B., and Alsberg, C. L. Preparation, solubility, and specific rotation of wheat gliadin. J. Biol. Chem. **65**: 279–304 (1925).

grown in the experimental fields of the Estação Agronómica Nacional, Sacavém, Portugal. From the results obtained, two extremes are presented in Table II, whose mean corresponds closely to the mean obtained from a larger series of determinations on the same sample. In Table II, the percentages of the amino acids, as well as the nitrogen, refer to the dry weight of protein.

The mean percentage of cystine-cysteine, of 2.83, is somewhat greater than the values stated in the literature. The author believes that the difference is due to his procedure permitting smaller losses.

Details of this work will be published elsewhere.

Acknowledgment

The author wishes to express his gratitude to Prof. J. A. Serra, Director of the Institute de Zoologia, University of Coimbra, for helpful criticism and facilities for the realization of the work.

A. Pereira
Estação Agronómica Nacional,
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October 15, 1951

BOOK REVIEWS

Flour for Man's Bread. A History of Milling. By John Storck and Walter Dorwin Teague, illustrated by Harold Rydell. xiii + 382 pp. University of Minnesota Press, Minneapolis, 1952. Price $7.50.

Millers have long been interested in the history of their industry, and justifiably so. For milling and its ancillary crafts have played a major role in the evolution of human society in many parts of the world, and led in the grand mechanization of industry a century and a quarter ago.

This volume grew out of the interest of James Ford Bell and his associates in the possibility of developing a museum of milling. Presently the services of the authors were devoted to the assembling and analysis of the great volume of literature and graphic records relating to milling that have been accumulated through the ages. This they have done effectively and well. They have traced the uses of cereals as food by primitive man, his early application of crude grinding appliances, of water, air and other sources of power, and, in due course, of many and elaborate automatic machines in the conversion of the seeds of certain grasses or grain into human food of superior quality.

The cosmopolitan nature of milling history becomes abundantly evident in the treatment here recorded. It began with primitive man, and was intimately associated with his determination to survive by storing food, such as cereals, for use during winter months and on his travels and military forays, and to improve the quality and delectability of his diet. Thus, men of many climes, races, and political persuasions contributed to the progress registered through the ages. Residents of America contributed mightily to the recent stages of development in milling technology, including Oliver Evans, La Croix, George T. Smith, and many others. Likewise, the millers of the United States were effectively alert in making early and useful application of many of the new principles and devices developed abroad.

The authors must have spent many busy hours in collecting all the factual material, and arranging it in such orderly and rational sequence for the convenience of the reader. They have been unusually skillful in their presentation of what might easily become a prosaic recital of engineering detail. The style of writing is attractive—in certain passages well-nigh poetic. Yet the essential factual material is in the text, in an attractive, readable setting. Moreover, the authors have appended not only citations to other sources of published material, which is a tribute to their erudition, but a vocabulary of milling terms which facilitates reading the technical portions of the text.

This review would not be complete without reference to the unique and artistic illustrations which adorn the text and render it more graphic. The artist, Harold Rydell, obviously grasped the spirit of the enterprise and made a substantial contribution thereto.

The last chapter "Looking Forward" constitutes an exciting challenge to the engineers and technologists of the future. The authors properly conclude that "we can only be sure that the story of milling has many chapters yet to be written; and we must wait until the future unfolds them, confident that they will be as profoundly significant in the record of human progress as any that can now be read."

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Baking Science and Technology. 2 volumes. By E. J. Pyler. 803 p. Siebel Publishing Company, Chicago, 1952. Price $15.00 per 2 volume set.

These volumes summarize the scientific and technical aspects of baking, placing the principal emphasis on the production of bread. They follow in the footsteps of the preceding editions of "Siebel's Manual for Bakers and Millers," which repre-

sented pioneering efforts in the dissemination of technical information on baking and milling. The original manual has been completely revised and the text is refreshing both because of the modern concept of technology and because of freedom from technical and mechanical errors.

The presentation of the subject matter follows a logical course of development, starting with purely theoretical considerations of basic science, progressing through a detailed description of baking material and ingredients to a discussion of their use in the production of bread, cakes, and other bakery foods. A final section is devoted to a discussion of the more important types of bakery equipment and sanitation.

Part I, Basic Science, consists of a brief but rather complete discussion of the carbohydrates, fats and proteins. These three introductory chapters are followed by chapters discussing enzymes (with special emphasis upon amylases and proteases), the vitamins, yeasts, molds and bacteria, and a final chapter concerning the aspects of physical chemistry. Readers who have had an elementary course in chemistry should have no difficulty in understanding the content of these chapters. Most practical bakers lack such a background and may consider the introductory chapters too abrupt and fail to comprehend their significance. It would appear, therefore, that a purely introductory chapter introducing the reader to the basic concepts of chemistry would greatly enhance their value to the practical baker. While the discussions are of necessity brief, the author has been quite liberal in literature citations so that the serious student can readily expand the basic information presented.

Having mastered Part I, the reader should have no difficulty in continuing to the subject matter of Part II. This section, Materials of Baking, includes chapters on wheat flour, miscellaneous flours, sugars, baking fats, milk and milk products, egg and egg products and water. The composition of these materials is discussed both in relationship to basic science and their function in the manufacture of the various bakery foods.

The "non-practical baker" will find Part III, Baking Technology, to be invaluable. The various phases of bread making, such as mixing, fermentation, make up, and the baking process are discussed in detail. The author has collected information from various sources and combined the material in a manner that is a pleasure to read. These chapters are followed by a discussion of rye bread production and bread staling. The section is completed by a chapter on physical and chemical testing methods. The author in this presentation discusses the origin of methods and their practical significance. The chapter is quite refreshing to those familiar with the stylized form of so many books of methods. It is perhaps unfortunate that a glossary of technical and practical terms was not included as these are frequently the cause of misunderstanding between the technically trained man and the practical baker.

Part IV concerns the Aspects of Cake Baking. Baking ingredients are reviewed with particular reference to their function in cake manufacture. The technology of cake baking is discussed in detail. It is in this section that the author discusses the physiology of both odor and taste, and the origin and processing of flavoring materials. The section is concluded by a chapter discussing the production of miscellaneous baked foods.

Part V, Bakery Equipment, is not equaled in any other publication. Equipment necessary for the operation of the modern bakery is described in detail, in the order of flour-receiving through the various stages of manufacture until the baked food is loaded for distribution to the retail outlets. The use of brand names and trademarks is somewhat unusual in technical publications but the author has dealt with a most difficult subject in a very satisfactory manner. The section is concluded by a chapter on bakery sanitation.

These volumes will meet a need long felt in the baking industry. Although, in this reviewer's opinion, they are of somewhat greater value to the technically trained man seeking knowledge of baking technology, the practical baker who is willing to put forth a little extra effort will find them extremely helpful. With introductory and supplementary material these volumes will serve as excellent text books for the study of baking technology. The volumes will be valued additions to any reference library.

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Biochemical Preparations. Vol. 2. By E. G. Ball, editor. vii + 109 pages. John Wiley and Sons, Inc., New York, N. Y., 1952. $3.00.

This is the second in a series of a collection of methods for synthesizing or isolating compounds of biochemical interest, volume I having been published 3 years previously. A total of 23 preparations is described, 18 of which are syntheses of biochemical compounds and 5 of which are isolation procedures. Each method is generally treated to include (1) the principle involved, (2) starting materials, (3) detailed procedure, (4) properties and purity of the product, and (5) a brief reference to other methods of preparation which are available. Each submitted procedure has been checked by an independent investigator, and the names of both submitter and checker are given. A particularly valuable feature of this book is the fact that detailed footnotes accompany the description of each method. Included in these footnotes are such valuable bits of information as suggested modification of the original procedure based on the experiences of the checker, the brand names of reagents and equipment, the feasibility of running larger batches than described in the text, and the lability of reagents and product. The methods of preparation are described in great clarity and with attention to detail which is not usually found in procedures published in biochemical journals.

More consideration might have been given to the isolation of enzymes. Crystalline lactate dehydrogenase is the only enzyme whose preparation is described in this volume. The mode of synthesis of organic compounds which are biochemically important can often be predicted on the basis of known chemical reactions, whereas methods of enzyme purification are largely empirical and hence the need for improved description and verification of details is greater. The value of this book might have been enhanced by the inclusion of a brief description of the biochemical importance of each compound whose preparation is described. For instance, it would have been both interesting and informative to know the biochemical significance of such compounds as DL-epi-Inosose-2 and phosphorylcholine without having to refer to other references for such information.

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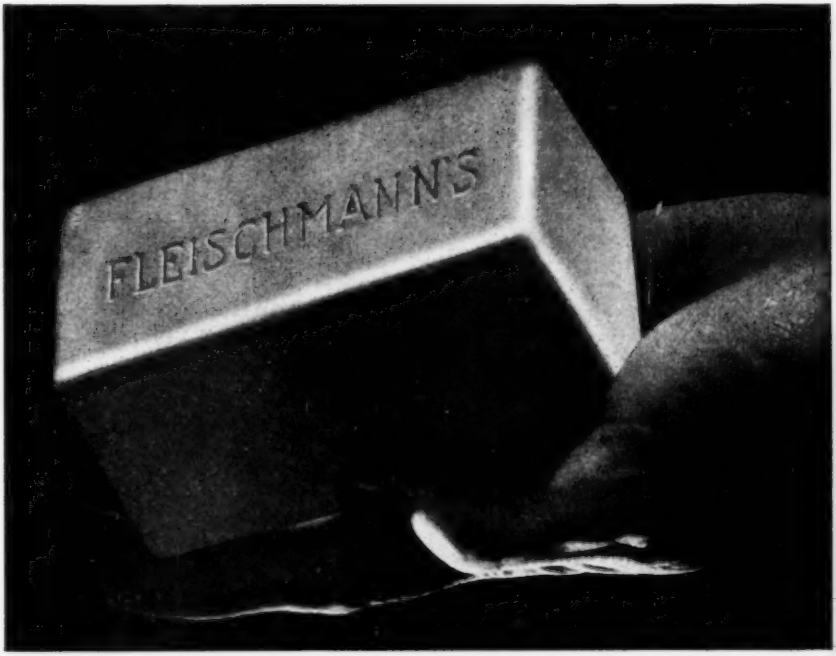

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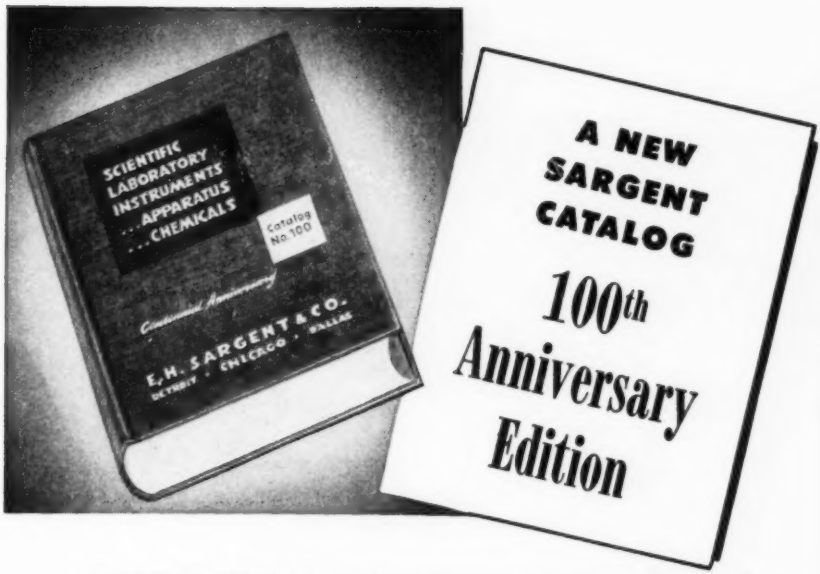

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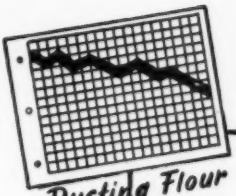

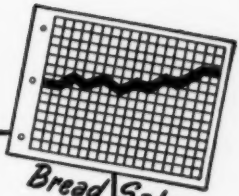

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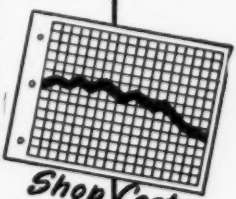

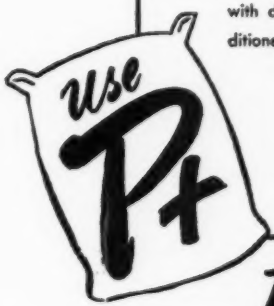

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